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 * W E L C O M E T O T H E *
 * U . S . P A T E N T T E X T F I L E *
 * * * * *

=> s nuclear localization and transfection
 49871 NUCLEAR
 5658 LOCALIZATION
 39 NUCLEAR LOCALIZATION
 (NUCLEAR(W) LOCALIZATION)
 1723 TRANSFECTION
 L1 18 NUCLEAR LOCALIZATION AND TRANSFECTION
 => d 1-18 kwic

US PAT NO: 5,496,731 [IMAGE AVAILABLE] L1: 1 of 18

SUMMARY:

BSUM(71)

In . . . proliferation of those cells. The expression vector is inserted into the abnormally proliferating cells by viral infection or transduction, liposome-mediated **transfection**, polybrene-mediated **transfection**, CaPO4 mediated **transfection** and electroporation. The treatment is repeated as needed.

DRAWING DESC:

DRWD(10)

FIG. . . . bladder tumor cells were transfected in multiple dishes with either p110.sup.RB (p.beta.A-f-RB33) or p94.sup.RB (p.beta.A-s-RB34) expression plasmids. Twenty-four hours after **transfection** the cells were labeled with [.sup.35 S]-methionine and chased with excess unlabeled methionine for 0, 6, 12 and 24 hours, . . .

DETDESC:

DETD(5)

The effects of **transfection** by either first or second in-frame AUG codon-initiated RB protein expression plasmid were compared on a number of well known. . .

DETDESC:

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DETD(29)

The . . . a plasmid or viral expression vector. A plasmid expression vector may be introduced into a tumor cell by calcium phosphate ****transfection****, liposome (for example, LIPOFECTIN)-mediated ****transfection****, DEAE Dextran-mediated ****transfection****, polybrene-mediated ****transfection****, electroporation and any other method of introducing DNA into a cell.

DETDESC:

DETD(48)

The . . . is administered in a composition comprising the vector together with a carrier or vehicle suitable for maintaining the transduction or ****transfection**** efficiency of the chosen vector and promoting a safe infusion. Such a carrier may be a pH balanced physiological buffer, . . .

DETDESC:

DETD(61)

Since non-functional mutations of the retinoblastoma protein are characterized by defects in phosphorylation, viral oncoprotein association and ****nuclear** localization**** (Templeton et al., 1991, Proc. Natl. Acad. Sci., USA, 88:3033-3037), the functional aspects of the artificial p94.sup.RB protein were studied. . .

DETDESC:

DETD(100)

For . . . DNA p.beta.A-s-RB34 (or pCMV-s-RB42) via LIPOFECTIN reagent (GIBCO BRL Life Technologies, Inc. Gaithersburg, Md.). Similar results have been obtained from ****transfection**** using calcium phosphate or electroporation methods.

DETDESC:

DETD(101)

The following procedures for ****transfection**** using LIPOFECTIN were modified from the manufacturer's specifications. Tumor cells were seeded in 100-mm dishes in appropriate growth medium supplemented. . . complex. While the lipid-DNA complex was forming, the cells were washed twice with 6 ml of serum-free medium. For each ****transfection****, 6 ml of

serum-free medium were added to each polystyrene tube containing the lipid-DNA complex. The solution was mixed gently,. . .

DETDESC:

DETD(105)

TABLE 2

Immunocytochemical Staining and [³ H] Thymidine Incorporation of RB-Defective Tumor Cells Following **Transfection** With p94.sup.RB or p110.sup.RB Expression Plasmids				
		Cells Incorporating		
Recipient	Protein	[³ H] Thymidine		
Cells	Promoter	Expressed	RB+	RB-

DETDESC:

DETD(107)

Approximately 48 hours after **transfection** the tumor cells were replated at a density of 10⁵ cells per 100 mm dish with selected medium containing G418. . .

DETDESC:

DETD(108)

Furthermore, . . . p94.sup.RB did suppress tumor cell growth. In contrast, 7 of 48 cell lines (approximately 15%) derived from tumor cells after **transfection** with the p110.sup.RB plasmid DNA were found to express the p110.sup.RB protein. This percentage was consistent with results expected in. . .

DETDESC:

DETD(111)

The HTB9 transfectants were also immunostained with MAb-1 anti-RB monoclonal antibody about 24 hours after **transfection**. The staining results are illustrated in FIG. 8.

DETDESC:

DETD(114)

Two . . . of G418-resistant colonies formed after treated with the plasmid victor pCMV-s-RB42 expressing p94.sup.RB, while no such effect was observed by ****transfection**** with the pCMV-f-RB35 plasmid (expressing p110.sup.RB protein). The difference was statistically significant (the two-tailed P values were less than 0.03. . . .

DETDESC:

DETD(118)

The . . . transfected in multiple dishes with either p110.sup.RB (FIG. 9, left) or p94.sup.RB (FIG. 9, right) expression plasmids. Twenty-four hours after ****transfection**** the cells were labeled with [³⁵S]-methionine and chased with excess unlabeled methionine for 0, 6, 12 and 24 hours,

DETDESC:

DETD(120)

The . . . and p.beta.A-f-RB33 (expressing p110.sup.RB, Section 4.3.5) or p.beta.A-s-RB34 (expressing p94.sup.RB Section 4.3.4) plasmid transfected 5637 cells approximately 24 hours after ****transfection****. The basic protocol for Western blot analysis was described in Xu, H-J., et al., 1989, Oncogene, 4:807-812. Each lane was. . . .

US PAT NO: 5,470,736 [IMAGE AVAILABLE]

L1: 2 of 18

SUMMARY:

BSUM(6)

Several . . . an alternatively spliced c-myb mRNA encodes a truncated form of the c-myb p75 which includes the DNA binding region and ****nuclear** **localization**** signal present in c-myb protein, but lacks regulatory regions required for transcriptional activation (Weber, et al., Science, 249:1291, 1990). The. . . .

DETDESC:

DETD(19)

When the host is a eukaryote, such methods of ****transfection**** of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, insertion of a plasmid encased in liposomes, or. . . .

DETDESC:

DETD(28)

Alternatively, . . . cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate ****transfection****. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral. . .

DETDESC:

DETD(69)

FosB2 . . . Briefly, embryonic carcinoma F9 cells were plated in 10 cm dishes at a density of 5.times.10.sup.6 cells/plate 24 hours before ****transfection****. ****Transfection**** protocol was as described (Chen, et al., Mol. Cell. Biol., 7:2745-2752, 1987) and .beta.-galactosidase activity was assayed by the CNPG. . . the reporter construct 5XTRECAT and 2 .mu.g of pBAG (a .beta.-galactosidase expression plasmid, which served as an internal control of ****transfection**** efficiency) were cotransfected into F9 cells with various combinations of expression plasmids.

DETDESC:

DETD(70)

For . . . reaction products were analyzed on TLC plates as described (Gorman, et al., Mol. Cell. Biol., 2:1044-1051, 1982). Forty-eight hours after ****transfection****, CAT activity was measured. All CAT activity assays were standardized with .beta.-galactosidase activity. Fold induction was standardized with the control. . .

DETDESC:

DETD(75)

208F . . . along with different amounts of a FosB2 expression plasmid (from 0-16 .mu.g); the total amount of DNA used in each ****transfection**** was kept constant by varying the amount of carrier DNA. Focus assays were performed as previously described (Miller, et al., Cell, 36:51-60, 1984). Each ****transfection**** was plated in duplicate. Foci were counted 12 days after ****transfection**** for v-Fos transfected cells, and 17 days after ****transfection**** for c-Fos and FosB transfected cells. FosB2 interfered with both the transcriptional transactivation and transformation potential of c-Fos and FosB, . . .

DETDESC:

DETD(87)

Ten . . . lines known in the art can alternately be used. The medium is changed 24 hours later and 48 hours after ****transfection****, the culture medium is harvested and used to infect the amphotropic packaging cell line .PSI.-CRIP, for example, in the presence. . .

US PAT NO: 5,468,624 [IMAGE AVAILABLE]

L1: 3 of 18

SUMMARY:

BSUM(3)

The . . . the steroid binding domain. This domain binds glucocorticoid to activate the receptor. This region of the receptor also has the ****nuclear**** ****localization**** signal. Deletion of this carboxyl terminal end results in a receptor that is constitutively active for gene induction (up to. . .

DRAWING DESC:

DRWD(2)

FIG. 1 shows the results of ****transfection**** of ICR 27 cells with GR constructs. ICR 27 cells were transfected with 4 different GR constructs, namely holo GR, . . . for cell kill in the absence (-) or presence (+) of 10^{-6} M dex for up to 96 hours after ****transfection****. The numbers above the boxes correspond to the amino acid position in the protein sequence of the steroid receptor. The . . . domain, respectively. The percentage reduction in viable cell number, both in the absence (-) or presence (+) of dex, following ****transfection**** of ICR 27 cells with these steroid receptor constructs is indicated. Superscript "a" indicates results for cell kill that are. . .

DRAWING DESC:

DRWD(3)

FIGS. . . . cell that is not transfected and on the right is a transfected cell. For all three cell lines, efficiency of ****transfection**** was about 40%.

DRAWING DESC:

DRWD(4)

FIG. . . . a control to measure percentage cell lysis or reduction in viable cell number, in the absence (-) of dex following **transfection**. Values given represent maximum kill observed. Maximum kill with or without dex occurred between 6-24 hr. Each **transfection** was done in triplicate.

DETDESC:

DETD(4)

Tests . . . in the range of 39%-51%, which is in agreement with the results of cell kill. Successive transfections of cells surviving **transfection** with glucocorticoid receptor constructs resulted in similar efficiencies of cell kill each time, confirming that electroporation was not merely eliminating. . . . took up the DNA. By blocking de novo protein and RNA synthesis, the onset of cell kill was arrested following **transfection** with an otherwise highly lethal constitutive receptor construct. Once the drug was removed and protein and RNA synthesis allowed to. . . .

DETDESC:

DETD(19)

The . . . (BioRad Laboratories, Richmond, Calif.) at 200 V, 500 .mu.F capacitance. The time constants or pulse time was recorded for each **transfection**--it ranged between 12 and 15 milliseconds. Five minutes after electroporating, the cells were resuspended to 4.times.10.sup.5 cells/ml in RPMI 1640. . . .

DETDESC:

DETD(20)

In experiments to verify the consistency of **transfection** efficiency, ICR 27 cells were electroporated three times in succession with glucocorticoid receptor constructs 465* and PRSHGRA. After each **transfection**, cell counts were determined soon after as well as 6, 12 and 24 hours after **transfection**. Cells were allowed to recover for 48 hours before being subject to a repeat pulse in the series of transfections.

DETDESC:

DETD(22)

At 24 hours after ****transfection****, cells were resuspended to 2×10^5 cells/ml in RPMI 1640 with 5% fetal bovine serum. When appropriate, dexamethasone (Sigma, St. Louis, . . .

DETDESC:

DETD(25)

Determining the Efficiency of ****Transfection****

DETDESC:

DETD(26)

In separate ****transfection**** experiments, ICR 27 cells were transfected by the protocol as described earlier with 15 μ g of P³²-labeled DNA, specifically PRSHGRA, 465* and 398-465*. Each ****transfection**** was done in duplicate. To label the DNA, the plasmids were digested with KpnI (to generate a 3' overhang), incubated. . . silver grains (above background i.e. >5 grains/cell) was recorded. Between 50-100 consecutive cells were counted per slide (4 slides per ****transfection****).

DETDESC:

DETD(28)

Preliminary . . . synthesis but not kill cells. ICR 27 cells were treated with cycloheximide or ethanol vehicle before electroporation and subsequently to ****transfection**** with 465* and pRShGR.alpha.. Cell counts and viability were recorded 15 minutes after, as well as 6, 12 and 19. .

DETDESC:

DETD(29)

In . . . 30 minutes prior to electroporation. The cells were washed with PBS and transfected. Cycloheximide or ethanol was readded subsequent to ****transfection****. At 30 minutes and 4-6 hours after ****transfection**** RNA and protein synthesis block was measured by incubating in the presence of the labeled precursors, TCA precipitating the products. . .

DETDESC:

DETD(31)

****Transfection**** of holo glucocorticoid receptor into

glucocorticoid-resistant ICR 27 cells could restore cell lysis on addition of 10^{-6} M dexamethasone (27,28,30). Since these were transient ****transfection**** assays, the extent of lysis was not 100%, but averaged $26 \pm 4\%$ in 23 assays, each done in triplicate. The holoreceptor. . .

DETDESC:

DETD(33)

A . . . construction is described earlier, was used to transfect ICR 27 cells as shown in FIG. 1. Within 6-24 hours of ****transfection**** and in the absence of dexamethasone 28% of the cells were lysed--an extent comparable to that evoked by the holoreceptor and steroid 48-96 hours after ****transfection****. Thus, a sequence which spans less than 100 amino acids is responsible for the constitutive lethality of the receptor. Although. . .

DETDESC:

DETD(34)

The following fragments or mutations of the GR gene were inactive for cell lysis upon ****transfection****: .DELTA.420-451 (deletion of the first zinc finger); .DELTA.450-487 (deletion of the second zinc finger); .DELTA.428-490 (deletion of the entire DNA. . .

DETDESC:

DETD(38)

Table 2 indicates the maximum percentage cell kill along with standard deviation values, seen in different cell lines, either after ****transfection**** with GR constructs 465* without $1 \mu\text{M}$ dex (in the second column) or the entire GR in the presence of. . .

DETDESC:

DETD(40)

The efficiency of ****transfection**** was determined in several experiments as all the assays were transient and no reporter gene had been co-transfected to serve as a control. ICR 27 cells were transfected with P.sup.32 -labeled constructs pRShGR.alpha., 465* and 398-465* in two separate ****transfection**** experiments. The labeled DNA in the cells was visualized by autoradiography. In the transfections with the holoreceptor between 40%-50% of. . . of cell kill varied between 22%-39% (27) for

these three glucocorticoid receptor constructs, which correlates well with the results of ****transfection**** efficiency, since it would be expected that a proportion of cells transfected would retain and express sufficient DNA to show. . .

DETDESC:

DETD(42)

To determine if similar fractions of cells would be killed if the cells surviving ****transfection**** are electroporated repeatedly, ICR 27 cells were electroporated three times in succession with 465* or the holoreceptor (as a control) as shown in FIG. 3. After each ****transfection****, cells were allowed to recover for 48 hours before being subjected to a repeat pulse. Similar fractions of cells were. . .

DETDESC:

DETD(44)

Cycloheximide . . . protein synthesis and/or RNA synthesis to determine if the lethal effects of 465* were specifically due to a product of ****transfection**** rather than ****transfection**** of the DNA itself into cells. The cell lethality kinetics of 465* are quick and therefore compatible with the maximum. . .

DETDESC:

DETD(46)

The . . . 21 missense amino acids and stops. It has some sequence important for binding GREs but lacks signals for transcriptional activation, ****nuclear**** ****localization****, steroid binding, and most sites for protein:protein interactions. It is constitutively active and can kill cells in which it is expressed within 6-24 hours of ****transfection****. The construct is as effective in effecting cell lysis as is the holoreceptor in the presence of steroid. Several of. . .

DETDESC:

DETD(47)

The efficiency of transient ****transfection**** assays was consistently in the range of 39%-51% irrespective of the gene construct used. These values are slightly higher than. . .

DETDESC:

DETD(48)

By repeatedly transfecting cells surviving ****transfection**** (three times in succession) similar killing efficiency was obtained and rules out the possibility that the transfections were wiping out. . .

US PAT NO: 5,449,755 [IMAGE AVAILABLE]

L1: 4 of 18

DETD(DESC):

DETD(3)

The . . . translation of a cyclin E mRNA. Antisense nucleic acids may be encoded within a host cell, e.g., following transduction or ****transfection**** of the cell with a vector DNA or RNA sequence encoding an antisense nucleic acid, or, alternatively, the antisense nucleic. .

DETD(DESC):

DETD(13)

Aspects . . . resistance markers, or markers satisfying the ,growth requirements of the cell. It will also be appreciated that in certain cells ****transfection**** or transduction with cyclin E nucleic acid will provide a selective proliferative/growth advantage that will serve as a type of. . .

DETD(DESC):

DETD(162)

Booher, . . . E., Hyams, J. S., and Beach, D. H. (1989). The fission yeast cdc2/cdc13/suc1 protein kinase: regulation of catalytic activity and ****nuclear** localization****. Cell 58, 485-497.

US PAT NO: 5,403,712 [IMAGE AVAILABLE]

L1: 5 of 18

DETD(DESC):

DETD(10)

Alternatively, . . . membrane protein which results in transport of the peptide into the cell. For localization to the nucleus one may use ****nuclear** localization**** sequences such as those defined for the glucocorticoid receptor or SV-40 large T antigen.

DETDESC:

DETD(44)

Transient **Transfection** Studies

DETDESC:

DETD(46)

For . . . duplicate or triplicate, and 2 .mu.g of the RSV-luciferase plasmid was included in each sample as an internal control for **transfection** efficiency. The total amount of DNA transfected into each sample of a given experiment was held constant by including an . . . an insert. CAT (Gorman et al., 1982) and luciferase (de Wet et al., 1987) activity were measured 36-48 hours after **transfection**. CAT assays were quantitated on an AMBIS radioactivity scanner, and the amount of CAT activity in each sample was corrected for **transfection** efficiency based on the results of the luciferase assay.

DETDESC:

DETD(51)

FIG. . . . bars) or presence (shaded bars) of DCoH expression vector. Cells were assayed for CAT activity 36 to 48 hours after **transfection**. Results are presented as the averages of duplicate measurements in a representative experiment and are corrected for **transfection** efficiency (Horton et al., 1989; Ho et al., 1989).

DETDESC:

DETD(73)

Testing . . . (Bodner et al., 1988; Ingraham et al., 1988) with DCoH did not enhance the amount of GHF-1-dependent CAT activity. Upon **transfection** of DCoH with the glucocorticoid receptor, enhancement of the ability of the glucocorticoid receptor to activate transcription of the MMTV. . . .

US PAT NO: 5,348,864 [IMAGE AVAILABLE]

L1: 6 of 18

DRAWING DESC:

DRWD(2)

FIG. . . . Highlighted domains include the leucine-rich domain (shaded box); the acidic region (black box) two proline-rich stretches (open box); two putative ****nuclear**** ****localization**** signals (left hatched box) and a cysteine-rich region (right hatched box).

DRAWING DESC:

DRWD(8)

FIG. . . . (Katzav, S. et al., supra); (b,c) nude mouse tumors induced by (b) second cycle- and (c) third cycle-transformants derived from ****transfection**** of NIH3T3 cells with human breast carcinoma DNA and (d) T24 human cells, were digested with Sac I and submitted. . .

DETDESC:

DETD(25)

Expression vectors may be introduced into host cells by various methods known in the art. For example, ****transfection**** of host cells with expression vectors can be carried out by the calcium phosphate precipitation method. However, other methods for. . .

DETDESC:

DETD(59)

Human . . . plasmids directed the synthesis of the expected vav protein as determined by immunoprecipitation analysis of G418-resistant NIH3T3 cells generated by co-****transfection**** of these plasmids with the selectable marker pSV2neo.

DETDESC:

DETD(63)

****Transfection**** of NIH3T3 cells, isolation of transformed cells, selection of G418-resistant colonies, metabolic labeling of cells with [³⁵S]-methionine, immunoprecipitation with. . .

DETDESC:

DETD(69)

Other . . . that may represent hinge regions; (iii) a putative protein kinase A phosphorylation site (residues 435 to 440); (iv) two

putative ****nuclear**** ****localization**** signals (residues 486 to 493 and 575 to 582); (v) a cysteine-rich sequence which includes two metal binding motifs Cys-X.sub.2. . .

DETDESC:

DETD(71)

Alignment . . . the putative protein kinase A phosphorylation site, the cystein-rich sequence that can fold into zinc finger-like structures and the putative ****nuclear**** ****localization**** signals, are also present in a mouse vav gene product (FIG. 2) [see SEQ. ID NO: 1]. The mouse vav. . .

DETDESC:

DETD(82)

****Transfection**** of NIH3T3 cells with pJC11 DNA, an expression plasmid carrying a mouse vav proto-oncogene, did not revealed significant levels of. . . that lacks 65 of the 67 amino-terminal residues absent in the human vav oncogene product (Katzav, S. et al., supra). ****Transfection**** of NIH3T3 cells with pJC12 DNA resulted in the appearance of about 3,000 foci of transformed cells per microgram of. . .

DETDESC:

DETD(83)

The . . . codon, translation from pJC7 DNA is likely to start in the second in-frame ATG, the initiator codon used by pJC12. ****Transfection**** of NIH3T3 cells with pJC25 DNA resulted in the appearance of about 40,000 foci of transformed cells per microgram of. . .

US PAT NO: 5,342,774 [IMAGE AVAILABLE]

L1: 7 of 18

DETDESC:

DETD(3)

In order to identify and isolate the gene coding for antigen PS15A, gene ****transfection**** was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line. . .

DETDESC:

DETD(6)

When . . . of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the **transfection** experiments.

DETDESC:

DETD(10)

Previous . . . 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library **transfection** to recover genes coding for tum.sup.- antigens.

DETDESC:

DETD(11)

The entire plasmid and genomic DNA of P1.HTR was prepared, following Wolfel et al., Immunogenetics 26: 178-187 (1987). The **transfection** procedure followed Corsaro et al., Somatic Cell Molec. Genet 7:603-616 (1981), with some modification. Briefly, 60 .mu.g of cellular DNA. . . flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after **transfection**, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350. . . .

DETDESC:

DETD(14)

Eight days after **transfection** as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-Paque. These. . . .

DETDESC:

DETD(25)

Using . . . supra, (60 ug) and 4 ug of pHMR272, described supra, groups of 5.times.10.sup.6 PO.HTR cells were used as transfectant hosts. **Transfection** was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group. . . .

DETDESC:

DETD(27)

As . . . 2274-2278 (1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, ****transfection**** and amplification of the cosmids followed example 5, again using PO.HTR.

DETDESC:

DETD(28)

High frequencies of ****transfection**** were observed, as described in Table 1, which follows:

DETDESC:

DETD(34)

This . . . was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after ****transfection****. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., . . .

DETDESC:

DETD(49)

Analysis . . . has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84:5306-5310 (1987), in a murine heterodomain protein having ****nuclear**** ****localization****.

DETDESC:

DETD(63)

The . . . shows phenotype H-2.sup.k. The cell lines were transfected with genes expressing one of the K.sup.d, D.sup.d, and L.sup.d antigen. Following ****transfection**** with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized. . .

DETDESC:

DETD(70)

In . . . conditions, and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high ****transfection****

frequency, i.e., it must be a "good" recipient.

DETDESC:

DETD(74)

Following . . . Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after **transfection**, the cells were harvested and seeded at 4.times.10.sup.6 cells per 80 cm.sup.2 flask in melanoma culture medium supplemented with 2. . . .

DETDESC:

DETD(76)

Thirteen days after **transfection**, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated. . . .

DETDESC:

DETD(83)

Cells . . . production as discussed in Example 17, supra. A total of 100 groups of E.sup.- cells (4.times.10.sup.6 cells/group) were tested following **transfection**, and 7.times.10.sup.4 independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected. . . .

DETDESC:

DETD(89)

The . . . preparation of a cosmid library. This library of nearly 50,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid **transfection** protocols described supra. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion. . . . fragments were cloned into vector pTZ 18, and then into MEL2.2. Again, TNF production was the measure by which successful **transfection** was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12. . . .

DETDESC:

DETD(134)

Cells . . . such as interleukins (e.g., IL-2 or IL-4), or major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules. Cytokine gene ****transfection**** is of value because expression of these is expected to enhance the therapeutic efficacy of the biologically pure culture of. . .

DETDESC:

DETD(135)

****Transfection**** with an MHC/HLA coding sequence is desirable because certain of the TRAs may be preferentially or specifically presented only by. . . particular MHC/HLA molecules. Thus, where a recipient cell already expresses the MHC/HLA molecule associated with presentation of a TRA, additional ****transfection**** is not necessary. On the other hand, it may be desirable to transfect with a second sequence when the recipient cell does not normally express the relevant MHC/HLA molecule. It is to be understood, of course, that ****transfection**** with one additional sequence does not preclude further ****transfection**** with other sequences.

DETDESC:

DETD(137)

****Transfection**** of cells requires that an appropriate vector be used. Thus, the invention encompasses expression vectors where a coding sequence for. . .

DETDESC:

DETD(138)

The . . . where each coding sequence is operably linked to a promoter. Again, the promoter may be a strong or differential promoter. Co-****transfection**** is a well known technique, and the artisan in this field is expected to have this technology available for utilization.

US PAT NO: 5,310,662 [IMAGE AVAILABLE]

L1: 8 of 18

SUMMARY:

BSUM(10)

It . . . sequences have identified certain functional domains within the molecule that are thought responsible respectively for DNA binding, hormone binding and ****nuclear** localization****. See Evans, et al.,

Science 240, 889 (1988) for a review of this subject matter. In the case of the. . .

DETDESC:

DETD(25)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including ****nuclear**** ****localization****, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by ****transfection**** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific. . .

DETDESC:

DETD(47)

DNA binding was measured as described previously (Hollenberg et al., supra). Mutant receptor, obtained in a crude COS-1 cell extract after ****transfection****, was incubated with a mixture of radiolabeled DNA fragments, one of which contained GRES. Receptor-DNA complexes were immunoprecipitated with receptor-specific. . .

DETDESC:

DETD(48)

****Transfection** and Luciferase Assays**

DETDESC:

DETD(49)

****Transfection**** of CV-1 and COS-1 cells was as described previously (Giguere et al., and Hollenberg et al., supra) using 5 micrograms. . .

DETDESC:

DETD(50)

Cell Culture and ****Transfection****

DETDESC:

DETD(51)

Conditions for growth and ****transfection**** of CV-1 (African green monkey kidney) cells were as previously described (Giguere et al., Cell 46, 645 (1986)), except that. . . Typically, 5 .mu.g reporter and 1 .mu.g expression vector were cotransfected, along with 2.5 .mu.g RSV-.beta.gal as a control for ****transfection**** efficiency. Acetylated and non-acetylated forms of [¹⁴]chloramphenicol were separated by thin layer chromatography, excised, and quantitated by liquid scintillation.

US PAT NO: 5,302,519 [IMAGE AVAILABLE]

L1: 9 of 18

SUMMARY:

BSUM(4)

The . . . dimerization domains. The bHLH family includes over 60 proteins in vertebrates, yeast, plants, and insects; many, if not all, exhibit ****nuclear** localization****, are sequence-specific DNA-binding proteins, and function as transcriptional regulators(6). The region of sequence similarity shared to Myc and other proteins. . .

DETDESC:

DETD(132)

Myc . . . Mad-1 to these proteins suggests that it should be localized to the nucleus as well. There is a potential bipartite ****nuclear** localization**** (Dingwall and Laskey, (1991) signal in Mad-1 found between amino acids 20 and 50. Myc and Max are in vivo. . .

DETDESC:

DETD(166)

In transient ****transfection**** assays, Myc activated transcription of a heterologous reporter gene containing the CACGTG binding motif in its promoter while Max repressed. . .

US PAT NO: 5,262,300 [IMAGE AVAILABLE]

L1: 10 of 18

SUMMARY:

BSUM(9)

It . . . sequences have identified certain functional domains within the molecule that are thought responsible respectively for DNA binding, hormone binding and ****nuclear** localization****. See Evans, et al., Science 240, 889 (1988) for a review of this subject matter. In the case of the . . .

DETDESC:

DETD(25)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including ****nuclear** localization****, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by ****transfection**** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific. . .

DETDESC:

DETD(48)

DNA binding was measured as described previously (Hollenberg et al., supra). Mutant receptor, obtained in a crude COS-1 cell extract after ****transfection****, was incubated with a mixture of radiolabeled DNA fragments, one of which contained GREs, Receptor-DNA complexes were immunoprecipitated with receptor-specific. . .

DETDESC:

DETD(49)

****Transfection** and Luciferase Assays**

DETDESC:

DETD(50)

****Transfection**** of CV-1 and COS-1 cells was as described previously (Giguere et al., and Hollenberg et al., supra) using 5 micrograms. . .

DETDESC:

=> s nuclear localization (p) transfection

49871 NUCLEAR

5658 LOCALIZATION

39 NUCLEAR LOCALIZATION
(NUCLEAR(W) LOCALIZATION)

1723 TRANSFECTION

L2 4 NUCLEAR LOCALIZATION (P) TRANSFECTION

=> d 1-4 kwic

US PAT NO: 5,468,624 [IMAGE AVAILABLE]

L2: 1 of 4

DETDESC:

DETD(46)

The . . . 21 missense amino acids and stops. It has some sequence important for binding GREs but lacks signals for transcriptional activation, ****nuclear** **localization****, steroid binding, and most sites for protein:protein interactions. It is constitutively active and can kill cells in which it is expressed within 6-24 hours of ****transfection****. The construct is as effective in effecting cell lysis as is the holoreceptor in the presence of steroid. Several of. . .

US PAT NO: 5,310,662 [IMAGE AVAILABLE]

L2: 2 of 4

DETDESC:

DETD(25)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including ****nuclear** **localization****, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by ****transfection**** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific.

US PAT NO: 5,262,300 [IMAGE AVAILABLE]

L2: 3 of 4

DETDESC:

DETD(25)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including ****nuclear** **localization****, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to

activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific.

US PAT NO: 5,217,867 [IMAGE AVAILABLE]

L2: 4 of 4

DETDESC:

DETD(24)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including **nuclear** **localization**, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific.

=> s nuclear localization (p) liposome

49871 NUCLEAR

5658 LOCALIZATION

39 NUCLEAR LOCALIZATION

(NUCLEAR(W)LOCALIZATION)

1746 LIPOSOME

L3 0 NUCLEAR LOCALIZATION (P) LIPOSOME

=> s nuclear localization (p) cationic lipid

49871 NUCLEAR

5658 LOCALIZATION

39 NUCLEAR LOCALIZATION

(NUCLEAR(W)LOCALIZATION)

41201 CATIONIC

10143 LIPID

19 CATIONIC LIPID

(CATIONIC(W)LIPID)

L4 0 NUCLEAR LOCALIZATION (P) CATIONIC LIPID

=> s poly l lysine and transfection

83059 POLY

452248 L

SEARCH ENDED BY USER

=> s poly l lysine (p) transfection

83059 POLY

452248 L

14346 LYSINE

484 POLY L LYSINE

(POLY(W)L(W)LYSINE)

1723 TRANSFECTION

L5 1 POLY L LYSINE (P) TRANSFECTION
=> d kwic

US PAT NO: 5,480,981 [IMAGE AVAILABLE]

L5: 1 of 1

SUMMARY:

BSUM(101)

Antisense . . . in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as ****poly**-(**L**-**lysine**)**. Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense. . . into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, CaPO.sub.4 -mediated DNA ****transfection****, electropotation, or other gene transfer vectors such as Epstein-Barr virus. Antisense or sense oligonucleotides are preferably introduced into a cell. . .

=> s poly l lysine (p) liposome

83059 POLY

452248 L

14346 LYSINE

484 POLY L LYSINE

(POLY(W)L(W)LYSINE)

1746 LIPOSOME

L6 5 POLY L LYSINE (P) LIPOSOME

=> d 1-5 kwic

US PAT NO: 5,508,184 [IMAGE AVAILABLE]

L6: 1 of 5

SUMMARY:

BSUM(27)

Therefore, . . . electroporation, possibly in conjunction with other processes for gene transfer used in microbiological research such as, for example, poly-L-ornithine or ****poly**-**L**-**lysine**** treatment, ****liposome**** fusion, DNA-protein complexing, charge modification on the protoplast membrane, fusion with microbial protoplasts or calcium phosphate correprecipitation and, especially, polyethylene. . .

US PAT NO: 5,453,367 [IMAGE AVAILABLE]

L6: 2 of 5

DETDESC:

DETD(42)

The . . . this step with techniques which are employed in microbiological research for gene transfer, for example by treatment with poly-L-ornithine or **poly**-*L**-*lysine**, **liposome** fusion, DNA protein complexing, altering the charge at the protoplast membrane, fusion with microbial protoplasts, or calcium phosphate co-precipitation and, . . .

US PAT NO: 5,231,019 [IMAGE AVAILABLE]

L6: 3 of 5

DETDESC:

DETD(41)

The . . . this step with techniques which are employed in microbiological research for gene transfer, for example by treatment with poly-L-ornithine or **poly**-*L**-*lysine**, **liposome** fusion, DNA protein complexing, altering the charge at the protoplast membrane, fusion with microbial protoplasts, or calcium phosphate co-precipitation and, . . .

US PAT NO: 4,921,757 [IMAGE AVAILABLE]

L6: 4 of 5

DETDESC:

DETD(20)

The . . . entrapped within the liposomes or packaged within the surrounding matrix. To prevent diffusion or release of the enzyme from the **liposome** or matrix, the molecular weight of the enzyme may be increased by any of a number of methods, thereby trapping. . . of substrate to protect the active site. Also, the enzyme could be covalently linked to a soluble polymer such as **poly**-*L**-*lysine**. The phospholipase is selected for ability to cleave one or more of the phospholipids making up the **liposome**, and non-toxicity to the organism or surrounding cells if for in vivo use.

US PAT NO: 4,900,556 [IMAGE AVAILABLE]

L6: 5 of 5

DETDESC:

DETD(20)

The . . . entrapped within the liposomes or packaged within the surrounding microcapsule. To prevent diffusion or release of the enzyme from the **liposome** or microcapsule, the enzyme molecular weight may be

increased by a number of methods, causing the enzyme to be trapped. . .
of substrate to protect the active site. Also, the enzyme could be
covalently linked to a soluble polymer such as **poly**-*L**-*lysine**.
The phospholipase is selected for ability to cleave one or more of the
phospholipids making up the **liposome**, and non-toxicity to the
organism or surrounding cells if for in vivo use.

=> s poly l lysine (p) cationic lipid

83059 POLY

452248 L

14346 LYSINE

484 POLY L LYSINE

(POLY(W)L(W)LYSINE)

41201 CATIONIC

10143 LIPID

19 CATIONIC LIPID

(CATIONIC(W)LIPID)

L7 0 POLY L LYSINE (P) CATIONIC LIPID

=> s dna binding (p) transfection

14427 DNA

81199 BINDING

501 DNA BINDING

(DNA(W)BINDING)

1723 TRANSFECTION

L8 26 DNA BINDING (P) TRANSFECTION

=> s dna binding domain (p) transfection

14427 DNA

81199 BINDING

21607 DOMAIN

76 DNA BINDING DOMAIN

(DNA(W)BINDING(W)DOMAIN)

1723 TRANSFECTION

L9 10 DNA BINDING DOMAIN (P) TRANSFECTION

=> d 1-10 kwic

US PAT NO: 5,468,624 [IMAGE AVAILABLE]

L9: 1 of 10

DRAWING DESC:

DRWD(2)

FIG. 1 shows the results of **transfection** of ICR 27 cells with GR
constructs. ICR 27 cells were transfected with 4 different GR constructs,
namely holo GR, . . . for cell kill in the absence (-) or presence (+)
of 10.sup.-6 M dex for up to 96 hours after **transfection**. The numbers
above the boxes correspond to the amino acid position in the protein
sequence of the steroid receptor. The. . . the left diagonal hatches,

the diamonds, the right diagonal hatches, and the chevrons correspond to the tau 1 domain, the ****DNA** **binding** **domain****, the tau 2 domain, and the steroid binding domain, respectively. The percentage reduction in viable cell number, both in the absence (-) or presence (+) of dex, following ****transfection**** of ICR 27 cells with these steroid receptor constructs is indicated. Superscript "a" indicates results for cell kill that are. . .

DETDESC:

DETD(31)

****Transfection**** of holo glucocorticoid receptor into glucocorticoid-resistant ICR 27 cells could restore cell lysis on addition of 10^{-6} M dexamethasone (27,28,30). Since these were transient ****transfection**** assays, the extent of lysis was not 100%, but averaged $26 \pm 4\%$ in 23 assays, each done in triplicate. The holoreceptor. . . dispensable for the cell lysis function. In fact, the present inventors have shown that construct .DELTA.9-385/532* which encodes the central ****DNA** **binding** **domain**** and sequences flanking it on either side could effectively lyse cells in a constitutive manner (27,28). However no previous tests had been done proving that neither the carboxyl-terminal end of the ****DNA** **binding** **domain**** nor the amino acids 1-8 and 385-397 could be eliminated with full retention of the lethal function. In order to further delineate the minimal sequence encoding this function, the present inventors deleted progressively from either end towards the central ****DNA** **binding** **domain****.

DETDESC:

DETD(33)

A . . . construction is described earlier, was used to transfect ICR 27 cells as shown in FIG. 1. Within 6-24 hours of ****transfection**** and in the absence of dexamethasone 28% of the cells were lysed--an extent comparable to that evoked by the holoreceptor and steroid 48-96 hours after ****transfection****. Thus, a sequence which spans less than 100 amino acids is responsible for the constitutive lethality of the receptor. Although. . . with partial deletions of the amino terminal domain of the GR always included parts of that region and the entire ****DNA** **binding** **domain****. That a sequence containing a ****DNA** **binding** **domain**** mutated in its 3' portion, and only the 22 proximal amino acids at the amino terminal end would be fully. . .

DETDESC:

DETD(34)

The following fragments or mutations of the GR gene were inactive for cell lysis upon ****transfection****: .DELTA.420-451 (deletion of the first zinc finger); .DELTA.450-487 (deletion of the second zinc finger); .DELTA.428-490 (deletion of the entire ****DNA** **binding** **domain****); I 422 (insertion of three amino acids between the first two cysteines in the first zinc finger); and GTG3A (chimeric GR having modified thyroid hormone receptor ****DNA** **binding** **domain**** which cannot recognize glucocorticoid response elements). Four point mutants having glycine substitutions in key amino acid positions in the zinc finger structure of the ****DNA** **binding** **domain**** were selected to test their importance in the cell kill process. The constructs transfected were G424, G442, G455 and G463--all . . .

DETDESC:

DETD(46)

The modified lethal fragment of the receptor, 398-465*, starts 23 amino acids upstream from the ****DNA** **binding** **domain****, goes through the first zinc finger, the linker region which follows it and through amino acid position 465 in the. . . for protein:protein interactions. It is constitutively active and can kill cells in which it is expressed within 6-24 hours of ****transfection****. The construct is as effective in effecting cell lysis as is the holoreceptor in the presence of steroid. Several of. . . zinc finger structure which binds the GREs in regulated genes. The mutants which had single amino acid substitutions in the ****DNA** **binding** **domain**** were functionally inactive for cell kill, stressing the importance of an intact ****DNA** **binding** **domain**** (at least halfway through the second zinc finger).

US PAT NO: 5,455,265 [IMAGE AVAILABLE]

L9: 2 of 10

DETDESC:

DETD(4)

Thus, . . . cells are grown in a medium during the first day of the assay detailed below as the "Cationic Liposome Mediated ****Transfection**** Assay". In the ****transfection**** procedure, which is performed during the second day of the ****transfection**** assay, the DNA plasmid coding for the CAT enzyme is also added to each cell culture, in addition to the. . . receptors (which were constructed in accordance with the teachings of the M. Pfahl et al. article) also contain a a ****DNA** **binding** **domain****, which is capable of binding to the "estrogen response element" (a DNA fragment) attached to the DNA plasmid coding for. . . to the ligand binding domain of the respective RAR.sub..alpha., RAR.sub..beta. etc.

receptor, only then is the receptor bound through its ****DNA****-****binding****
****domain**** to the estrogen response element of the estrogen-response-
element-chloramphenicol-acetyl transferase-construct (ERE-CAT). In other
words, through multiple interactions CAT enzyme is manufactured. . .

US PAT NO: 5,446,150 [IMAGE AVAILABLE]

L9: 3 of 10

DETDESC:

DETD(117)

Construction . . . and gel-purified. This fragment was then
substituted into the vector pRRSfokIR to replace the DNA segment coding
for the FokI ****DNA****-****binding**** ****domain**** and, hence, form the
Ubx-F.sub.N hybrid gene (FIG. 17B). After ****transfection**** of competent
RR1 cells with the ligation mix, several clones were identified by
restriction analysis and their DNA sequences were. . .

US PAT NO: 5,440,586 [IMAGE AVAILABLE]

L9: 4 of 10

DETDESC:

DETD(4)

Thus, . . . cells are grown in a medium during the first day of the
assay detailed below as the "Cationic Liposome Mediated ****Transfection****
Assay". In the ****transfection**** procedure, which is performed during the
second day of the ****transfection**** assay, the DNA plasmid coding for the
CAT enzyme is also added to each cell culture, in addition to the. . .
receptors (which were constructed in accordance with the teachings of the
M. Pfahl. et al. article) also contain a ****DNA**** ****binding****
****domain****, which is capable of binding to the "estrogen response
element" (a DNA fragment) attached to the DNA plasmid coding for. . .
to the ligand binding domain of the respective RAR.sub..alpha.,
RAR.sub..beta. and receptor, only then is the receptor bound through
its ****DNA****-****binding**** ****domain**** to the estrogen response element of
the estrogen-response-element-chloramphenicol-acetyl transferase-
construct (ERE-CAT) capable of initiating transcription of messenger RNA
for the CAT. . .

US PAT NO: 5,440,662 [IMAGE AVAILABLE]

L9: 5 of 10

DETDESC:

DETD(25)

The . . . a series of events and protein-protein interactions that

ultimately result in activation. If more than one essential function is encoded by the DNA** **binding** **domain**, some of the non-functional point mutants may still retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific.

US PAT NO: 5,742,077 [IMAGE AVAILABLE]

L9: 6 of 10

DETDESC:

DETD(109)

FIG. . . . transferred to the expression vector pRS (see Giguere, et al., (1986), and the Not1/Xho1 restriction fragment of pRShGR.sub.NX containing the DNA** **binding** **domain** was introduced into pRShRnx between the Not1 and Xho1 sites to create pRShRGR. B, Cell **transfection** and CAT assay. The recombinant DNA constructs (5 .mu.g each) were introduced into CV-1 cells by calcium phosphate coprecipitation.

US PAT NO: 5,762,300 [IMAGE AVAILABLE]

L9: 7 of 10

DETDESC:

DETD(25)

The . . . allosteric events and protein-protein interactions that ultimately result in activation. If more than one essential function is encoded by the DNA** **binding** **domain**, some of the non-functional point mutants may still retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific.

US PAT NO: 5,777,867 [IMAGE AVAILABLE]

L9: 8 of 10

DETDESC:

DETD(24)

The . . . allosteric events and protein-protein interactions that ultimately result in activation. If more than one essential function is encoded by the DNA** **binding** **domain**, some of the non-functional point mutants may still retain their ability to bind DNA but fail to

activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific.

US PAT NO: 5,171,671 [IMAGE AVAILABLE]

L9: 9 of 10

DETDESC:

DETD(109)

FIG. . . . to the expression vector pRS (see Giguere, et al., (1986), and the Not1/Xho1 restriction fragment of pRShGR.sub.NX containing the hGR **DNA**-**binding** **domain** was introduced into pRShRnx between the Not 1 and Xho1 sites to create pRShRGR. B, Cell **transfection** and CAT assay. The recombinant DNA constructs (5 .mu.g each) were introduced into CV-1 cells by calcium phosphate coprecipitation (see. . . .

US PAT NO: 4,981,784 [IMAGE AVAILABLE]

L9: 10 of 10

DETDESC:

DETD(114)

FIG. . . . to the expression vector pRS (see Giguere, et al., (1986), and the Not1/Xho1 restriction fragment of pRShGR.sub.NX containing the hGR **DNA**-**binding** **domain** was introduced into pRShRnx between the Not1 and Xho1 sites to create pRShRGR. B, Cell **transfection** and CAT assay. The recombinant DNA constructs (5 .mu.g each) were introduced into CV-1 cells by calcium phosphate coprecipitation (see. . . .

=> s dna binding domain (p) liposome

14427 DNA

81199 BINDING

21607 DOMAIN

76 DNA BINDING DOMAIN

DNA(W) BINDING (W) DOMAIN)

1746 LIPOSOME

L10 2 DNA BINDING DOMAIN (P) LIPOSOME

=> d 1-2 kwic

US PAT NO: 5,468,265 [IMAGE AVAILABLE]

L10: 1 of 2

DETDESC:

DETD(4)

Thus, . . . that HeLa cells are grown in a medium during the first

day of the assay detailed below as the "Cationic **Liposome** Mediated Transfection Assay". In the transfection procedure, which is performed during the second day of the transfection assay, the DNA. . . receptors (which were constructed in accordance with the teachings of the M. Pfahl et al. article) also contain a a **DNA** **binding** **domain**, which is capable of binding to the "estrogen response element" (a DNA fragment) attached to the DNA plasmid coding for. . . to the ligand binding domain of the respective RAR.sub..alpha., RAR.sub..beta. etc. receptor, only then is the receptor bound through its **DNA**--**binding** **domain** to the estrogen response element of the estrogen-response-element-chloramphenicol-acetyl transferase-construct (ERE-CAT). In other words, through multiple interactions CAT enzyme is manufactured. . .

US PAT NO: 5,599,586 [IMAGE AVAILABLE]

L10: 2 of 2

DETDESC:

DETD(4)

Thus, . . . that MeLa cells are grown in a medium during the first day of the assay detailed below as the "Cationic **Liposome** Mediated Transfection Assay". In the transfection procedure, which is performed during the second day of the transfection assay, the DNA. . . receptors (which were constructed in accordance with the teachings of the M. Pfahl. et al. article) also contain a a **DNA** **binding** **domain**, which is capable of binding to the "estrogen response element" (a DNA fragment) attached to the DNA plasmid coding for. . . to the ligand binding domain of the respective RAR.sub..alpha., RAR.sub..beta. etc. receptor, only then is the receptor bound through its **DNA**--**binding** **domain** to the estrogen response element of the estrogen-response element-chloramphenicol-acetyl transferase-construct (ERE-CAT) capable of initiating transcription of messenger RNA for the CAT.

=> s dna binding domain (p) cationic lipid

1442' DNA

81199 BINDING

2160' DOMAIN

7' DNA BINDING DOMAIN

DNA (W) BINDING (W) DOMAIN)

41201 CATIONIC

1014' LIPID

2' CATIONIC LIPID

(CATIONIC(W)LIPID)

L11 7' DNA BINDING DOMAIN (P) CATIONIC LIPID

=> logoff y

U.S. Patent & Trademark Office LOGOFF AT 11:50:29 ON 24 APR 96

TI 11q23 translocations split the "AT-hook" cruciform DNA-binding region and the transcriptional repression domain from the activation domain of the mixed-lineage leukemia (MLL) gene.

AU Zeleznik-Le N J; Harden A M; Rowley J D

CS Department of Medicine, University of Chicago, IL 60637..

NC CA42557 (NCI)

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Oct 25) 91 (22) 10610-4.

Journal code: PV3. ISSN: 0027-8424.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 9501

AB Translocations involving chromosome band 11q23, found in acute lymphoid and myeloid leukemias, disrupt the MLL gene. This gene encodes a putative transcription factor with homology to the zinc fingers and other domains of the Drosophila trithorax gene product and to the "AT-hook" motif of high mobility group proteins. To map potential transcriptional activation or repression domains of the MLL ***protein***, yeast GAL4 ***DNA*** - ***binding*** ***domain*** and MLL hybrid protein-expressing plasmids were cotransfected with chloramphenicol acetyltransferase reporter plasmids in a transient ***transfection*** system. We found that MLL contains a strong activation domain and a repression domain. The former, located telomeric (3') to the breakpoint region, activated transcription 18-fold to > 200-fold, depending on the promoter and cell line used for ***transfection***. A repression domain that repressed transcription 4-fold was located centromeric (5') to the breakpoint region of MLL. The MLL AT-hook domain protein was expressed in bacteria and was utilized in a gel mobility shift assay to assess DNA-binding activity. The MLL AT-hook domain could bind cruciform DNA, recognizing structure rather than sequence of the target DNA. In translocations involving MLL, loss of an activation domain with retention of a repression domain and a DNA-binding domain on the der(11) chromosome could alter the expression of downstream target genes, suggesting a potential mechanism of action for MLL in leukemia.

L11 ANSWER 6 OF 15 MEDLINE

AN 94286555 MEDLINE

TI Positive and negative transcriptional control by the TAL1 helix-loop-helix protein.

AU Hsu H L; Wadman I; Tsan J T; Baer R

CS Department of Microbiology, University of Texas Southwestern Medical Center, Dallas 75235..

NC CA46593 (NCI)

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES
 OF AMERICA, (1994 Jun 21) 91 (13) 5947-51.
 Journal code: PV3. ISSN: 0027-8424.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 9409
 AB Tumor-specific activation of the TAL1 gene is the most common
 genetic defect associated with T-cell acute lymphoblastic leukemia.
 The TAL1 gene products possess a basic helix-loop-helix (bHLH)
 motif, a ***protein*** -dimerization and ***DNA*** -
 binding ***domain*** found in several transcription
 factors. TAL1 polypeptides interact, in vitro and in vivo, with
 class A bHLH proteins (e.g., E47) to form heterodimers with
 sequence-specific DNA-binding activity. In this study, we show that
 TAL1 can regulate the transcription of an artificial reporter gene
 that contains binding sites for bHLH heterodimers involving TAL1.
 Transcription of the reporter is strongly induced by E47-E47
 homodimers and moderately induced by TAL1-E47 heterodimers. Thus, in
 a cellular environment that allows formation of E47-E47 homodimers
 (e.g., in the absence of Id regulatory proteins) TAL1 can repress
 transcription by recruiting E47 into bHLH complexes with less
 transcriptional activity (i.e., TAL1-E47 heterodimers). However, in
 other settings TAL1 can activate transcription because TAL1-E47
 heterodimers are more resistant to negative regulation by Id
 proteins. Hence, TAL1 can potentially regulate transcription in
 either a positive or negative fashion.

L11 ANSWER 7 OF 15 MEDLINE
 AN 94067166 MEDLINE
 TI Mechanism of glucocorticoid induction of the rat plasminogen
 activator inhibitor-1 gene in HTC rat hepatoma cells: identification
 of cis-acting regulatory elements.
 AU Bruzdinski C J; Johnson M R; Goble C A; Winograd S S; Gelehrter T D
 CS Department of Human Genetics, University of Michigan Medical School,
 Ann Arbor 48109-0618..
 NC CA-22729 (NCI)
 AM-07245 (NIADDK)
 T32-GM-07544 (NIGMS)
 SO MOLECULAR ENDOCRINOLOGY, (1993 Sep) 7 (9) 1169-77.
 Journal code: NGZ. ISSN: 0888-8809.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 9403

AB Type 1 plasminogen activator inhibitor (PAI-1) is the major physiological inhibitor of plasminogen activation, inhibiting both tissue- and urokinase-type plasminogen activators. In HTC rat hepatoma cells, glucocorticoids increase PAI-1 activity, antigen and mRNA accumulation 3- to 5-fold; this increase is due solely to an increase in the rate of PAI-1 gene transcription. We have identified the cis-acting sequences in the 5'-flanking sequence of the HTC PAI-1 gene that mediate this induction. Analysis of a series of hybrid genes containing various portions of the PAI-1 5'-flanking region fused to the chloramphenicol acetyltransferase reporter gene transfected into HTC cells localized the region involved in the transcriptional regulation by glucocorticoids to between -1237 and -764. Electrophoretic mobility shift assays and DNase-I protection assays showed that a glucocorticoid response element (GRE) 15-mer located at -1212 bound the glucocorticoid receptor ***DNA*** - ***binding*** ***domain*** ***protein*** in a concentration-dependent manner. Mutations created within this GRE eliminated its ability both to confer a glucocorticoid response and to bind the glucocorticoid receptor. When placed upstream of a heterologous promoter in either orientation, this GRE conferred glucocorticoid inducibility. We, therefore, conclude that the sole cis-acting sequence required for the glucocorticoid response of the PAI-1 gene in rat HTC hepatoma cells is the GRE at -1212.

L11 ANSWER 8 OF 15 MEDLINE

AN 93181242 MEDLINE

TI The DNA binding domain of the varicella-zoster virus gene 62 protein interacts with multiple sequences which are similar to the binding site of the related protein of herpes simplex virus type 1.

AU Tyler J K; Everett R D

CS MRC Virology Unit, Glasgow, UK..

SO NUCLEIC ACIDS RESEARCH, (1993 Feb 11) 21 (3) 513-22.

Journal code: O8L. ISSN: 0305-1048.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 9306

AB Varicella-zoster virus gene 62 encodes a protein with predicted Mr of 140,000D (VZV 140k) that shares extensive predicted amino acid sequence homology with the major immediate early (IE) transcriptional regulator protein of herpes simplex virus type 1 (HSV-1) Vmw175. The integrity of highly conserved region 2 is essential for the DNA binding and transcriptional regulatory functions of Vmw175. Similarly, an insertion mutation in region 2 (codons 468-641) of 140k eliminates the transcriptional repression and activation functions of this protein. We have expressed a

fragment of 140k which encompasses region 2 as a non-fusion polypeptide in bacteria. This 140k ***DNA*** ***binding*** ***domain*** ***peptide*** (codons 417-646) binds to numerous DNA sequences throughout the VZV gene 62 promoter region. It induces multiple regions of protection from DNase I digestion, flanked by sites of DNase I hypersensitivity. Several of the sites recognized can be considered to be divergent forms of the consensus sequence which is recognized by Vmw175. However, by use of a panel of mutagenized probe fragments, we found that the 140k DNA binding domain was less sequence-specific than Vmw175 in its interactions with DNA. Consistent with this, the homologous Vmw175 DNA binding domain, and also intact Vmw175, recognize the gene 62 binding sites much less efficiently than the 140k DNA binding domain. Also in contrast to the situation with Vmw175, the 140k DNA binding domain failed to induce DNA bending when occupying the binding sites in its own promoter. Deletion analysis has mapped the minimal DNA binding domain of the VZV 140k protein, as measured in gel retardation analysis, to lie within residues 472 to 633. The differences in binding characteristics of the DNA binding domains of the homologous VZV 140k and HSV-1 Vmw175 IE proteins may account for the subtle differences in their regulatory activities in ***transfection*** assays and during virus growth in tissue culture.

L11 ANSWER 9 OF 15 MEDLINE
 AN 92380993 MEDLINE
 TI Localization of O-GlcNAc modification on the serum response transcription factor.
 AU Reason A J; Morris H R; Panico M; Marais R; Treisman R H; Haltiwanger R S; Hart G W; Kelly W G; Dell A
 CS Department of Biochemistry, Imperial College of Science, Technology, and Medicine, London, United Kingdom..
 NC HD 13563 (NICHD)
 CA 42486 (NCI)
 HD 13563 (NICHD)
 +
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Aug 25) 267 (24) 16911-21.
 Journal code: HIV. ISSN: 0021-9258.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 9212
 AB A unique form of nucleoplasmic and cytoplasmic protein glycosylation, O-linked GlcNAc, has previously been detected, using Gal transferase labeling techniques, on a myriad of proteins (for review see Hart, G. W., Haltiwanger, R. S., Holt, G. D., and Kelly, W. G. (1989a) Annu. Rev. Biochem. 58, 841-874), including many RNA

polymerase II transcription factors (Jackson, S. P., and Tjian, R. (1988) Cell 55, 125-133). However, virtually nothing is known about the degree of glycosylation at individual sites, or, indeed, the actual sites of attachment of O-GlcNAc on transcription factors. In this paper we provide rigorous evidence for the occurrence and locations of O-GlcNAc on the c-fos transcription factor, serum response factor (SRF), expressed in an insect cell line. Fast atom bombardment mass spectrometry (FAB-MS) of proteolytic digests of SRF provides evidence for the presence of a single substoichiometric O-GlcNAc residue on each of four peptides isolated after sequential cyanogen bromide, tryptic, and proline specific enzyme digestion: these peptides are 306VSASVSP312, 274GTTSTIQTAP283, 313SAVSSADGTVLK324, and 374DSSTDLTQTSSSGTVTLTP391. Using an array of techniques, including manual Edman degradation, aminopeptidase, and elastase digestion, together with FAB-MS, the major sites of O-GlcNAc attachment were shown to be serine residues within short tandem repeat regions. The highest level of glycosylation was found on the SSS tandem repeat of peptide (374-391) which is situated within the transcriptional activation domain of SRF. The other glycosylation sites observed in SRF are located in the region of the ***protein*** between the ***DNA*** ***binding*** ***domain*** and the transcriptional activation domain. Glycosylation of peptides (274-283) and (313-324) was found to occur on the serine in the TTST tandem repeat and on serine 316 in the SS repeat, respectively. The lowest level of glycosylation was recovered in peptide (306-312) which lacks tandem repeats. All the glycosylation sites identified in SRF are situated in a relatively short region of the primary sequence close to or within the transcriptional activation domain which is distant from the major sites of phosphorylation catalyzed by casein kinase II.

L11 ANSWER 10 OF 15 MEDLINE
 AN 92372658 MEDLINE
 TI ***DNA*** - ***binding*** ***domain*** of RCC1
 protein is not essential for coupling mitosis with DNA replication.
 AU Seino H; Hisamoto N; Uzawa S; Sekiguchi T; Nishimoto T
 CS Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan..
 SO JOURNAL OF CELL SCIENCE, (1992 Jul) 102 (Pt 3) 393-400.
 Journal code: HNK. ISSN: 0021-9533.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 9211
 AB The RCC1 protein that is required for coupling mitosis with the S

phase has a DNA-binding domain in the N-terminal region outside the repeat. We found that RCC1 protein without any DNA-binding activity complemented the tsBN2 mutation with the same efficiency as that of intact RCC1 protein. In ts+ transformants of tsBN2 cells transfected with the RCC1 cDNA lacking the DNA-binding domain, an endogenous RCC1 disappeared at 39.5 degrees C, and the deleted RCC1 protein encoded by the transfected cDNA was found in the cytoplasm, but a significant amount of it was also found in the nuclei. This deleted RCC1 protein was eluted from the nuclei with the same concentration of NaCl and DNase I as was used for the intact RCC1 protein in BHK21 cells. Furthermore, the deleted RCC1 protein co-migrated with the nucleosome fraction on sucrose density gradient analysis. These results indicate that the RCC1 protein binds chromatin with the aid of other unknown protein(s). Thus, the ***DNA*** - ***binding*** ***domain*** of RCC1 ***protein*** is not essential for coupling between the S and M phases, but was shown instead to function as a nuclear translocation signal.

L11 ANSWER 11 OF 15 MEDLINE

AN 90356384 MEDLINE

TI The lack of transcriptional activation of the v-erbA oncogene is in part due to a mutation present in the ***DNA*** - ***binding*** ***domain*** of the ***protein*** .

AU de Verneuil H; Metzger D

CS Laboratoire de Genetique Moleculaire des Eucaryotes du CNRS, Unite 184 de Biologie Moleculaire, Faculte de Medecine, Strasbourg, France..

SO NUCLEIC ACIDS RESEARCH, (1990 Aug 11) 18 (15) 4489-97.
Journal code: O8L. ISSN: 0305-1048.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 9011

AB Using a transient co- ***transfection*** system we have demonstrated that response elements for estrogen (ER), thyroid hormone (TR) and retinoic acid receptors (RAR) are closely related. Thyroid hormone-induced activation of transcription was observed in CV1 cells and not in HeLa cells, suggesting the existence of cell-specific transcription factors necessary for the response. By contrast to its cellular counterpart (c-erbA/cTR alpha) the oncogene protein gag v-erbA is unable to activate gene transcription from different response elements derived from the rat growth hormone (rGH) gene promoter. A chimeric construct consisting of the ER in which the DNA binding domain has been replaced by that of cTR alpha was able to stimulate the reporter gene. In contrast, a construct in which ER DNA binding domain has been replaced by that of gag v-erbA

did not activate gene transcription. These results lead us to the conclusion that the mutated DNA binding domain of v-erbA is in part responsible for the lack of transcriptional activation and in repression of gene expression. This is due in large part to the Gly73----Ser mutation which corresponds to the position of one of the three discriminating amino acids that are thought to interact with a specific base of the response element.

L11 ANSWER 12 OF 15 MEDLINE

AN 90128258 MEDLINE

TI Activation of transcription by v-myb: evidence for two different mechanisms.

AU Klempnauer K H; Arnold H; Biedenkapp H

CS Zentrum fur Molekulare Biologie, Universita Heidelberg, FRG..

SO GENES AND DEVELOPMENT, (1989 Oct) 3 (10) 1582-9.

Journal code: FN3. ISSN: 0890-9369.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 9005

AB The retroviral oncogene v-myb encodes a nuclear, sequence-specific DNA-binding protein. To investigate the possibility that v-myb encodes a transcriptional regulator, we used a transient cotransfection assay to explore the potential of v-myb to influence the expression of other genes. We found that expression of a chicken lysozyme promoter/CAT gene construct was activated by v-myb in the presence of myb-specific binding sites. Action was not observed with a truncated v-myb ***protein*** lacking its ***DNA*** - ***binding*** ***domain***. We also observed that expression of a hybrid human HSP70 promoter/CAT gene, lacking myb-specific binding sites, was activated by v-myb. However, in this case, the truncated v-myb protein, which lacked its DNA-binding domain, also activated HSP70/CAT expression, indicating that trans-activation of this gene construct was independent of the sequence-specific DNA-binding activity of the v-myb protein. These observations suggest that v-myb encodes a trans-activator and that activation of gene expression occurs by two different mechanisms, one of which involves specific binding of v-myb protein to the regulated gene.

L11 ANSWER 13 OF 15 MEDLINE

AN 89311606 MEDLINE

TI Functional characterization of a complex ***protein*** - ***DNA*** - ***binding*** ***domain*** located within the human immunodeficiency virus type 1 long terminal repeat leader region.

AU Malim M H; Fenrick R; Ballard D W; Hauber J; Bohnlein E; Cullen B R

CS Howard Hughes Medical Institute, Duke University Medical Center,
Durham, North Carolina 27710..
SO JOURNAL OF VIROLOGY, (1989 Aug) 63 (8) 3213-9.
Journal code: KCV. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 8910
AB Transcriptional trans activation of the human immunodeficiency virus
type 1 (HIV-1) long terminal repeat (LTR) by the viral tat trans
activator is mediated by an LTR-specific sequence located
immediately 3' to the start of transcription initiation. We have
used a range of molecular techniques to examine DNA-protein
interactions that occur in the vicinity of this cis-acting sequence.
Our results demonstrate the existence of a sequence-specific
DNA-protein interaction involving the HIV-1 leader DNA and map this
binding event to between -2 and +21 base pairs relative to the HIV-1
LTR transcription start site. Evidence suggesting that this
interaction involves three distinct protein-DNA contact sites
extending along one side of the DNA helix is presented. Mutation of
these sites was found to ablate protein-DNA binding yet was observed
to have no effect on either the basal or tat trans-activated level
of HIV-1 LTR-specific gene expression. We therefore conclude that
this DNA-protein interaction has a function distinct from the
regulation of HIV-1 LTR-specific gene expression.

L11 ANSWER 14 OF 15 MEDLINE
AN 89261819 MEDLINE
TI Functional domains of the human vitamin D3 receptor regulate
osteocalcin gene expression.
AU McDonnell D P; Scott R A; Kerner S A; O'Malley B W; Pike J W
CS Department of Cell Biology, Baylor College of Medicine, Houston,
Texas 77030..
NC AR-38170
DK-38130
HD-07857
SO MOLECULAR ENDOCRINOLOGY, (1989 Apr) 3 (4) 635-44.
Journal code: NGZ. ISSN: 0888-8809.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 8909
AB The human vitamin D receptor (VDR) has been cloned recently. Two
cDNAs comprising the full-length VDR were spliced, cloned into a
mammalian expression vector, and transiently expressed in COS-1

cells. The protein product exhibited properties consistent with that observed for receptor in human cells. A series of 5'- and 3'-deletions of the full-length VDR cDNA was prepared and evaluated. Native DNA binding was localized to a peptide fragment (residues 1-114) whose most prominent feature is the cysteine rich region proven to represent the DNA binding domain in other steroid receptors. Steroid binding-competence required synthesis of a peptide that initiated C-terminal to the DNA-binding domain at residue 114 and which contained the remaining 313 residues. To determine the location of elements within the receptor necessary for transcription, an osteocalcin gene promoter-chloramphenicol acetyltransferase reporter gene was cotransfected together with wild type or mutant VDR cDNAs and the latter's effect on chloramphenicol acetyltransferase activity was assessed. Cotransfection of wild type receptor alone resulted in efficient transcription of the reporter plasmid. However, synthesis of a ***peptide*** containing the ***DNA*** ***binding*** ***domain*** as well as 76 residues carboxy terminal to this region exhibited some degree of activity, albeit constitutive. These results suggest that the functional domains of the VDR are similar to that of other steroid receptors and that these domains participate in the transcriptional regulation of the human osteocalcin gene.

L11 ANSWER 15 OF 15 MEDLINE
 AN 89072761 MEDLINE
 TI Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets.
 AU Hughes M R; Malloy P J; Kieback D G; Kesterson R A; Pike J W; Feldman D; O'Malley B W
 CS Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030..
 SO SCIENCE, (1988 Dec 23) 242 (4886) 1702-5.
 Journal code: UJ7. ISSN: 0036-8075.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 8903
 AB Hypocalcemic vitamin D-resistant rickets is a human genetic disease resulting from target organ resistance to the action of 1,25-dihydroxyvitamin D3. Two families with affected children homozygous for this autosomal recessive disorder were studied for abnormalities in the intracellular vitamin D receptor (VDR) and its gene. Although the receptor displays normal binding of 1,25-dihydroxyvitamin D3 hormone, VDR from affected family members has a decreased affinity for DNA. Genomic DNA isolated from these families was subjected to oligonucleotide-primed DNA amplification,

and each of the nine exons encoding the receptor protein was sequenced for a genetic mutation. In each family, a different single nucleotide mutation was found in the ***DNA*** ***binding*** ***domain*** of the ***protein*** ; one family near the tip of the first zinc finger (Gly----Asp) and one at the tip of the second zinc finger (Arg----Gly). The mutant residues were created in vitro by oligonucleotide directed point mutagenesis of wild-type VDR complementary DNA and this cDNA was transfected into COS-1 cells. The produced protein is biochemically indistinguishable from the receptor isolated from patients.

=> s (protein or peptide) (2a) dna binding domain and liposome

```

593672 PROTEIN
155363 PEPTIDE
380545 DNA
357469 BINDING
42934 DOMAIN
2161 DNA BINDING DOMAIN
      (DNA(W) BINDING(W) DOMAIN)
111 (PROTEIN OR PEPTIDE) (2A) DNA BINDING DOMAIN
3760 LIPOSOME
L12      0 (PROTEIN OR PEPTIDE) (2A) DNA BINDING DOMAIN AND LIPOSOME

```

=> s (protein or peptide) (2a) dna binding domain and cationic lipid?

```

593672 PROTEIN
155363 PEPTIDE
380545 DNA
357469 BINDING
42934 DOMAIN
2161 DNA BINDING DOMAIN
      (DNA(W) BINDING(W) DOMAIN)
111 (PROTEIN OR PEPTIDE) (2A) DNA BINDING DOMAIN
6579 CATIONIC
141113 LIPID?
      77 CATIONIC LIPID?
          (CATIONIC(W) LIPID?)
L13      0 (PROTEIN OR PEPTIDE) (2A) DNA BINDING DOMAIN AND CATIONIC
          LIPID?

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FULL ESTIMATED COST	27.10	27.25

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FILE 'HOME' ENTERED AT 11:54:42 ON 24 APR 96

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FULL ESTIMATED COST	0.15	0.15

FILE 'MEDLINE' ENTERED AT 11:54:51 ON 24 APR 96

FILE LAST UPDATED: 22 APR 1996 (960422/UP). FILE COVERS 1966 TO DATE.
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*** DETAILS. ***

=> s nuclear localization and transfection

```

130587 NUCLEAR
68403 LOCALIZATION
11800 NUCLEAR LOCALIZATION
      (NUCLEAR (W) LOCALIZATION)
29982 TRANSFECTION
L1    196 NUCLEAR LOCALIZATION AND TRANSFECTION

```

=> s nuclear localization signal and transfection

```

130587 NUCLEAR
68403 LOCALIZATION
79190 SIGNAL
30400 NUCLEAR LOCALIZATION SIGNAL
      (NUCLEAR (W) LOCALIZATION (W) SIGNAL)
29982 TRANSFECTION
L2    196 NUCLEAR LOCALIZATION SIGNAL AND TRANSFECTION

```

=> s (peptide or protein) and nuclear localization signal and transfection

1553: PEPTIDE

5936: PROTEIN

1305: NUCLEAR

684: LOCALIZATION

791: SIGNAL

35: NUCLEAR LOCALIZATION SIGNAL

(NUCLEAR (W) LOCALIZATION (W) SIGNAL)

299: TRANSFECTION

LE (PEPTIDE OR PROTEIN) AND NUCLEAR LOCALIZATION SIGNAL AND T
TRANSFECTION

=> d 1-10 bil a)

LF ANSWER OF 64 MEDLINE

AN 96102146 MEDLINE

TL An amino acid sequence motif sufficient for subnuclear localization
of an arginine/serine-rich splicing factor.

AU Hedley D L; Amrein H; Maniatis T

CS Department of Molecular and Cellular Biology, Harvard University,
Cambridge, MA 02138, USA.

NC GM42231 (N MS)

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES
OF AMERICA (1995 Dec 5) 92 (25) 11524-8.

Journal code: PV3. ISSN: 0027-8424.

CY United States

DT Journal Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 9603

AB We have identified an amino acid sequence in the Drosophila
Transformer (Tra) ***protein** that is capable of directing a
heterologous ***protein*** to nuclear speckles, regions of the
nucleus previously shown to contain high concentrations of
spliceosome small nuclear RNA and splicing factors. This sequence
contains a nucleoplasmin-like bipartite ***nuclear***
localization ***signal*** (NLS) and a repeating
arginine/serine (RS) dipeptide sequence adjacent to a short stretch
of basic amino acids. Sequence comparisons from a number of other
splicing factors that colocalize to nuclear speckles reveal the
presence of one or more copies of this motif. We propose a two-step
subnuclear localization mechanism for splicing factors. The first
step is transport across the nuclear envelope via the
nucleoplasmin-like NLS, while the second step is association with

component in the speckled domain via the RS dipeptide sequence.

LE ANSWER OF 64 MEDLINE

AN 960790 MEDLINE

TI Efficient nuclear localization and immortalizing ability, two functions dependent on the adenovirus type 5 (Ad5) E1A second exon, are necessary for cotransformation with Ad5 E1B but not with T24ras.

AU Douglas J L; Quinlan M P

CS Department of Microbiology and Immunology, University of Tennessee Health Science Center, Memphis 38163, USA.

NC CA-505 (DOI)

SC JOURNAL OF VIROLOGY, (1995 Dec. 69 (12) 8061-5.

Journal Code: KCV. ISSN: 0022-538X.

CY United States

DT Journal Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EN 9603

AB Expression of adenovirus type 5 E1A 12S is sufficient to immortalize primary rat kidney cells, but another viral or cellular oncogene such as E1B or T24ras, is necessary for complete transformation. The regions of 12S sufficient for T24ras cotransformation have been well characterized and are located in the first exon. The second exon is dispensable for ras cotransformation, although it contains a region which appears to modulate the transformation phenotype. The same 12S first exon regions important in ras transformation are also necessary for E1B transformation. Analysis of an extensive series of second exon deletion and amino acid point mutations demonstrated that mutations affecting either the efficient nuclear localization and/or the immortalizing ability of the ***protein*** also prevented cooperation with E1B. In general, the entire terminal half of 12S, including the ***nuclear*** ***localization*** ***signal***, was necessary for efficient cotransformation with E1B. In addition to the differences between T24ras and E1B regarding 12S regions necessary for cotransformation, the characteristics of E1B-cotransformed foci differed from those of T24ras. The E1B foci took longer to appear and had a much slower growth rate. No hyperplastic foci were produced with E1B cotransfections, and established E1A-E1B lines exhibited minimal growth in soft agar compared to that of E1A-T24ras lines.

LE ANSWER OF 64 MEDLINE

AN 96074 MEDLINE

TI Mutational analysis of p80 co1 in indicates a functional interaction between ribbed bodies and the nucleolus.

AU Bohman J; Ferreira J; Leonard A I

CS European Molecular Biology Laboratory, Heidelberg, Germany..

SC JOURNAL OF CELL BIOLOGY, (1995 Nov) 131 (4) 817-31.

Journal of Cell Biology: HMV. ISSN: 0021-5525.

CY United States

DI Journal Article; (JOURNAL ARTICLE)

LA English

FS Prior Journals; Cancer Journals

EM 9602

AB Coiled bodies are conserved subnuclear domains found in both plant and animal cells. They contain a subset of splicing snRNPs and several molecular antigens, including Nopp140 and fibrillarin. In addition, autoimmune patient sera have identified a coiled body specific ***protein*** called p80 coilin. In this study we show that p80 coilin is ubiquitously expressed in human tissues. The full-length human p80 coilin ***protein*** correctly localizes in coiled bodies when exogenously expressed in HeLa cells using a transient ***transfection*** assay. Mutational analysis identifies separate domains in the p80 coilin ***protein*** that differentially affect its subnuclear localization. The data show that p80 coilin has a ***nuclear*** ***localization*** ***signal***, but this is not sufficient to target the ***protein*** to coiled bodies. The results indicate that localization in coiled bodies is not determined by a simple motif analogous to the NLS motifs involved in nuclear import. A specific carboxy-terminal deletion in p80 coilin results in the formation of pseudo-coiled bodies that are unable to recruit splicing snRNPs. This results in a loss of endogenous coiled bodies. A separate class of mutant p80 coilin proteins are shown to localize in fibrillar structures that surround the nucleoli. These mutants also lead to loss of endogenous coiled bodies, produce a dramatic disruption of nucleolar architecture and cause a specific segregation of nucleolar antigens. The structural change in nucleoli is accompanied by the loss of RNA polymerase II activity. These data indicate that p80 coilin plays an important role in subnuclear organization and suggest that there may be a functional interaction between coiled bodies and nucleoli.

LE ANSWER OF 14 MEDLINE

AN 960697 MEDLINE

TI Initiation of Transcription: A Factor that binds to the transcription initiation site of the histone h5 gene, is a glycoprotein member of a family of cell growth regulators [corrected] published elsewhere appears in Mol Cell Biol 1996 Feb;16(2):500-510.

AU Gonzalez-Blanco A; Martinez-Moncal M; Ruiz-Carrillo A

CS Cancer Research Center, Medical School of Laval University, 1100 Ste. Julie de Quebeck, Quebec

SC MOLECULAR CELL BIOLOGY, (1995 Dec) 15 (12) 6670-85.

Journal: *NGY* (1995) 17:306.
 CY: *Unif. J. Biol. Sci.*
 DT: *Journal of Molecular Biology* (1995) 271:1-10 (CLE)
 LA: *English*
 FS: *Prior. J. Biol. Sci.*
 EM: 9603
 AF: Initiation of transcription [corrected] (IBR) is a chicken erythroid factor (approx. molecular mass, 70 to 73 kDa) that binds to a sequence spanning the transcription initiation site of the hemoglobin gene, repressing its transcription. A variety of other cells, including transformed erythroid precursors, do not have IBR but a factor referred to as IBF (68 to 70 kDa) that recognizes the same sites. We have cloned the IBR cDNA and studied the relationship of IBR and IBF. IBR is a 503-amino-acid-long acidic protein which is 94% identical to the recently reported human α -1-2 globulin factor and highly related to the invertebrate transcription factor, P3A1 and erected wing gene product (EWG). We present evidence that IBR and IBF are most likely identical proteins differing in their degree of glycosylation. We have analyzed several nuclear aspects of IBR/F and shown that the factor exists as a stable dimer and that the dimer is the relevant DNA-binding protein. The evolutionarily conserved N-terminal half of IBR/F harbors the DNA-binding/dimerization domain (ouster 127 to 283), one or several casein kinase II sites (37 to 100) and a bipartite nuclear localization signal (89 to 100) which appears to be necessary for nuclear targeting. Binding site analysis revealed that the alternating RCGTGGY consensus sequence is a high-affinity IBR/F binding site and that the direct repeat sequence TGCGCATGCGCA is the optimal site. A survey of genes potentially regulated by this family of factors identified genes involved in growth-related metabolism.

LE: *ANSWER* (1995) 17:306.
 AN: 9603
 T1: The two isoforms of mouse terminal deoxynucleotidyl transferase differ in both the ability to add N regions and subcellular localization.
 AU: Benoit M; Fardoux J; Nguyen Q T; Martinez O; Rougeon F; Doyen P.
 CS: *Unif. J. Biol. Sci.* (1995) 17:306-310, Paris Cedex 15, France.
 SC: *EMBO J.* (1995) 14:4221-9.
 DT: *Journal of Molecular Biology* (1995) 271:1-10 (CLE).
 CY: *English*
 DT: *Journal of Molecular Biology* (1995) 271:1-10 (CLE)
 LA: *English*

FS Prior Journals

EM 1501

At Two alternative splicing of terminal deoxynucleotidyl transferase transferase (TdT) and TdT-L code respectively for proteins of 119 and 129 amino acids have been previously identified in the mouse thymus. Here we show that the same two transcripts are also present in B-lymphocyte cells from bone marrow. In addition we demonstrate that the cytosolic tailing of terminal deoxynucleotidyl transferase found near the carboxy-terminal end of TdT significantly alters the function of the enzyme. In contrast to TdT, TdT-L does not catalyse N region insertion at the junction of a V(D)J site-specific recombinational substrate. In an attempt to explain the lack of N region insertions we have characterized the different parameters which distinguish the two isoforms of TdT. Examination of transcribed cell extracts revealed a reduced capacity of TdT-L to add nucleotides to the 3' end of DNA, consistent with a lower terminal transferase activity. Moreover, the half-life of the TdT-L ***protein*** in these extracts is 2-fold shorter than that of TdT. Finally, despite the fact that TdT-L has the same ***nuclear*** ***localization*** as TdT, the cellular localization of the two isoforms was strikingly different. In contrast to nuclear TdT, TdT-L was found exclusively in the cytoplasm. These characteristics could contribute to the functional differences between the two isoforms of TdT. However, the subcellular localization of TdT-L on its own can account for its inability to add N region

LE ANSWER TO PRIOR JOURNALS

AP 1995 1501 JEDN

TE The last 50 amino acids of the C-terminal domain of human p53 is dispensable for both transcriptional regulation and inhibition of tumor cell growth.

AL Pellegrini M, et al. Cancer Res 55: 1171-1176 (1995)

CS Department of Microbiology and Molecular Genetics, University of California, San Francisco, CA 94143

NO CA 94143

CA 94143

SC ONCOGENE 95 (1995) 337-49.

Journal of Cell Biology 130: 1-12 (1995)

CY ENGLAND: Oxford University Press

DE Journal of Cell Biology 130: 1-12 (1995)

LE English

FS Priority of Journal of Cell Biology

EM 1501

At To investigate the role of the C-terminal domains of the human p53 protein in its growth suppressive and transcriptional properties deletion mutants were constructed which delete p53 delta 363, 60 (p53 delta 333)

depends on the presence of specific sequences (LZ1 to LZ3) and a carboxy-terminal response element (LZ4) as well as on the presence of a titratable factor in the cell. The activation of hHSF1 appears to induce hHSF1 binding to the DNA. We have used these results to uncover an amino-terminally located domain that is responsible for the *****localization***** *****signal*****. A mechanism involving the controlling oligomerization regulates the transcriptional activity of hHSF1. Components of this mechanism were mapped to a region, including LZ2 and nearby sequences downstream of LZ1, that is clearly separated from the carboxy-terminal transcription activation domain(s). We propose that this region forms a feedback structure that masks the transcriptional activity of hHSF1 in the unstressed cell but is opened up by modification of hHSF1 and/or binding of a factor facilitating hHSF1 unfolding in the stressed cell. Activation of hHSF1 appears to intervene at several independently regulated structural transitions.

L3 21 MAR 8 OJ *****localization*****
 AN 28 015 *****localization*****
 TI Molecular cloning of a novel transcription factor that controls stromelysin gene expression.
 AU Gonzalez; Moscatelli; Hernandez T
 CS Centro de Biología Celular y Molecular Severo Ochoa (Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid), Spain..
 SC J Biol Chem 270 (1995 Jun) 15 (6) 3164-70.
 CY United States
 DT Journal; Article
 LA English
 FS Biology Journal
 OS GENE, UK-UL
 EM
 AB Stromelysin, like the metalloproteinases with the widest substrate specificity, plays a critical role in tumor invasion and metastasis. Hernandez et al. reported an element (SPRE) of the stromelysin gene that lies between nucleotides -1221 and -1203 and is necessary for the control of stromelysin gene expression. This element, which induces a nuclear activity that binds to the SPRE, being a concatenated probe with several copies of the SPRE, to screen a lambda gt11 cDNA expression library from mouse 3T3 fibroblasts, we report here the molecular cloning and sequencing for a novel *****protein***** (pSPBP) of stromelysin that binds to this element and has several features of a transcription factor, such as a putative leucine zipper region and a *****localization***** *****signal***** domain with homology to the DNA-binding domain of SPBP. The fact that SPBP is at least a critical component of the SPRE nuclear binding activity is

AN 1988 J Biol Chem 263:11448-11454
 TI Nuclear localization signal in the hnRNP A1 ***protein***
 AU Simon M; Drabek J
 CS Drabek J; Simon M; University of Pennsylvania School
 of Medicine, Philadelphia 19104-6148, USA..
 SO JOURNAL OF BIOLOGICAL CHEMISTRY (May) 129 (3) 551-60.
 CY United States
 DT Journal; Article (JOURNAL ARTICLE)
 LA English
 FS Laboratory Journals
 EM 1988
 AB Heterogeneous nuclear ribonucleoprotein (hnRNP) A1 ***protein*** is one of the major protein-RNA binding proteins in eukaryotic cells and one of the most abundant proteins in the nucleus. It is localized to the nucleoplasm and also shuttles between the nucleus and the cytoplasm. The amino acid sequence of A1 contains two RNP motifs (RNA binding domain) at the amino terminus and a glycine-rich domain at the carboxyl terminus. This configuration, designated 2x1 domain, is representative of perhaps the largest family of hnRNP proteins. Unlike hnRNP proteins characterized so far, A1 (and most 2x1 RBD proteins) does not contain a recognizable ***nuclear*** localization signal*** (NLS). We have found that a segment of 40 amino acids near the carboxyl terminus (designated M9) is necessary and sufficient for nuclear translocation; attaching this segment to the cytoplasmic protein beta-galactosidase or to pyruvate kinase completely translocated these otherwise cytoplasmic proteins to the nucleus. The isolated another RNA binding motif found in the glycine-rich domain, the RBD box, are not required for A1 nuclear localization. This novel type of nuclear localization domain as it does not resemble similar to classical basic-type NLS. Interestingly, the amino acids near to M9 are found in other nuclear RNA binding proteins including hnRNP A2.

=> d his

HOME ENCL 1 (1988) (ON 24 APR 96)

FILE MEDLINE 1988 (1988) (ON 24 APR 96)

L1 1988 JOURNAL OF BIOLOGICAL CHEMISTRY AND TRANSFECTION
 L2 72 JOURNAL OF BIOLOGICAL CHEMISTRY AND TRANSFECTION
 L3 64 JOURNAL OF BIOLOGICAL CHEMISTRY AND NUCLEAR LOCALIZATION SIGNAL AN

=> s 1988 JOURNAL OF BIOLOGICAL CHEMISTRY AND NUCLEAR LOCALIZATION SIGNAL AND TRANSFECTION

1. 363 P

2. 672 P

3. 587 N

4. 403 L

5. 197 S

6. 338 N

7. 48 (

8. 982 T

L4 3 (

=> d 1-3 b b ab

L4 ANSWER 1 OF

AN 15065 62

TI Mutational analysis of murine Mx1 protein: GTP binding core domain is essential for antiviral activity.

AF Melen K; Juliano

CS Molecular Biology Program, National Public Health Institute, Helsinki, Finland

SO VIROLOGY, (1996) 205 (2), 269-79.

Journal code: JAL 1996: 0001-6822.

CY United States

DT Journal; Article (JOURNAL ARTICLE)

LA English

FS Infectious Diseases - General Journals

EM 1502

AB Interferon-induced resistance to influenza virus in murine cells is mediated by the Mx1 protein, which inhibits viral mRNA synthesis in the nucleus. Mx1 protein is a GTPase specifically targeted into the cell nucleus and has a C-terminal leucine zipper domain that mediates dimerization. In order to determine functionally important elements of the protein we created several substitution, insertion, and deletion mutants of murine Mx1 protein. The antiviral activity of mutant Mx1 proteins was analyzed by a transient transfection ** /influenza A infection assay in COS cells by measuring virus fluorescence. Mx1 proteins carrying mutations in the GTP binding element showed reduced, but not completely lost, antiviral activity. Mutations produced, GTP binding element substitution mutants showed very low (< 10%) GTPase activity as measured by type Mx1 protein. Mutations in other portions of the protein had less effect on antiviral activity, except one mutation which was situated six amino acids from the C-terminal and which evidently interrupted the

lear* ... ***signal rendering
 the ***prot ... and clearly reduced the
 anti-influenza activity of various sizes and locations
 further suggest that the C-terminal half of the molecule is more
 important in its anti-influenza activity than other regions of the
 molecule. These results indicate that the GTP binding domain of Mx1
 protein is essential for its anti-influenza activity, correlating to
 the low GTPase activity of the GTP binding element substitution
 mutants, but other parts of the molecule such as the leucine
 zipper and the hydrophobic signal are of importance, too.

LE ANSWER 2 OF ...
 AT 92050 03 ...
 TI ... for the activity of p53 protein.
 AU Shaulsky G; Golan G; ... Levine A J; Rotter V
 CS Department of Cell Biology, Weizmann Institute of Science, Rehovot,
 Israel.
 SO COGNETE, (C. A. North) (1995) 65.
 JURN cod ... 5-9232.
 CM ENGLAND: Un ...
 DT ...
 LA ...
 FS ...
 EM ...
 AB p53 appears to be a growth regulator, the perturbation of which
 induces changes in cell proliferation. Wild-type p53 protein
 is thought to function as a growth arrest gene, whereas mutant p53,
 which accumulates in cancer cells, has been shown to enhance
 malignant transformation. To test if wild-type and mutant p53 migrate into
 the cell nucleus by different identical nuclear localization signals
 (LS) inherent in their primary sequences. Results presented here
 show that the suppressive activity of wild-type p53 measured as the
 reduction of anchorage dependence of primary rat fibroblasts induced by
 oncogenic transformation with ras and either E1A or mutant p53, as
 well as the stimulatory effect of mutant p53 estimated by
 cooperation with ras in the formation of primary rat fibroblasts,
 is dependent upon nuclear localization signals in p53 protein. While
 transfection of unmodified wild-type p53 significantly
 reduces the number of anchorage-dependent fibroblast-transformed foci
 induced by E1A, ras and p53, the wild-type p53
 protein with mutated LS has almost lost this suppressive activity.
 Partially defective mutant p53, with a reduced nuclear
 accumulation capability, still inhibits some suppressive activity. In
 addition, wild-type p53 coding for intact mutant p53
 protein efficiently cooperates with the ras oncogene, whereas the
 corresponding wild-type p53 are totally inert. On this basis
 we conclude that a functional LS is essential for both wild-type and mutant

p53 is a fundamental feature for manifesting the activities of these proteins. Both the suppressor activity mediated by the wild-type p53 and the enhancement of tumorigenicity mediated by the mutant p53 require nuclear localization of the proteins to function.

LA ANSWER 3 OF 1 MEL 1

AT 91216452 MEDLINE

TI Targeting the Escherichia coli lac repressor to the mammalian cell nucleus.

AF 1; Hill; David

CO Division of Biology, California Institute of Technology, Pasadena 91125.

SO GENETICS (1991 Jun 15) Vol. 134 (2) 141-50.
Journal code: GENETICS 134 2 1419.

CY Netherlands

ED Journal; Author; Journal Article

LA English

PS Priority Journal

EA 9108

AB We have previously shown that about 90% of total Escherichia coli lac repressor synthesized in mammalian cells is located in the cytoplasm [Muller and Hill, Cell 48 (1987) 555-566]. To target a functional repressor to the nucleus, we mutated 10 nucleotides at the 3' end of the coding sequence, thus adding the nuclear localization signal of the human virus 40 large-T antigen to the C terminus of the repressor. The mutant lacI gene and the wild-type (wt) gene, each in a plasmid animal cell expression vectors, driven by the promoter of the 20S adenovirus long terminal repeat, were transfected into several rodent cell lines. In confirmation of our previous results, only about 10% of the wt repressor, but all of the mutant repressor, was localized in the nucleus. DNase I footprint analyses showed that the mutant repressor retained the same operator DNA-binding specificity as the wt repressor. Furthermore, both repressor-operator complexes could be dissociated by addition of poly(ethyl-hexyl)-carbazole or dextran sulfate in vitro. However, the ratio of number of repressor monomers per nucleus that, by in vitro assay, could bind to the operator sequence to the number of monomer repressor polypeptides per nucleus, as determined by Western blotting, was about 1:1 for the wt repressor and about 1:30 for the mutant repressor. These results indicate: (a) the mutant repressor assembles less efficiently on the DNA; and/or (b) it has reduced binding affinity to the operator sequence; and/or (c) it has higher binding affinity to cytoplasmic DNA.

AB nuclear localization; repressor; and liposome

1 58717 1R

68403 L 11128 A

79197 SIGNAL

338 L 11128 A 11128 A SIGNAL

11128 A 11128 A 11128 A (N(W) SIGNAL)

760 L 11128 A

11128 A 11128 A 11128 A 11128 A AND LIPOSOME

= d b a b

11128 A 11128 A 11128 A 11128 A

21128 A 11128 A 11128 A 11128 A

71128 A 11128 A 11128 A 11128 A mediated gene transfer into
molecular tissue by replication deficient adenovirus.

21128 A 11128 A 11128 A 11128 A; Chapman G D; Naftilan A J

01128 A 11128 A 11128 A 11128 A University, Nashville, Tennessee,
21128 A 11128 A 11128 A 11128 A

11128 A 11128 A 11128 A 11128 A

11128 A 11128 A 11128 A 11128 A

50128 A 11128 A 11128 A 11128 A 11128 A 11128 A

Journal code: 11128 A 11128 A 11128 A 11128 A

01128 A 11128 A 11128 A 11128 A

11128 A 11128 A 11128 A 11128 A 11128 A 11128 A

11128 A 11128 A 11128 A 11128 A

11128 A 11128 A 11128 A 11128 A

11128 A 11128 A 11128 A 11128 A

21128 A 11128 A 11128 A 11128 A experiments, especially in vascular
cells an efficient method of gene transfer into this
tissue would be a valuable tool. Previous methods have either
yielded low levels of gene transfer or require complicated manipulation
of viral vectors. The purpose of this study was to develop an easy,
efficient method for introducing unmodified plasmid DNA into vascular
tissue. In this report it is demonstrated that complexing unmodified
plasmid DNA with replication deficient adenovirus (Ad5 dl312) via
cationic liposomes results in a 1000-fold increase in gene transfer up to 1000-fold in cultured
endothelial cells (ECs). Further, utilizing a
karyon-injected cell model, intense nuclear
staining was observed in smooth muscle cell layer and the
arterial wall. Following transfection with a plasmid containing
the lacZ gene and the SV40 promoter, nuclear
localization of the lacZ gene was demonstrated no detectable
staining was observed. This complexing plasmid DNA with
adenovirus results in a 1000-fold increase in gene transfer both in vivo
and in vitro. This method could have a wide range of applications
in the experimental vascular system.

= s r d e r l o c a t i o n g e n e t r a n s f e r l i p i d ?

DOES THE ORDER OF ADDITION OF ANIONIC AND CATIONIC LIPID?

... d l ...

[illegible]

101 14.37 2 (12.5) 100
 102 14.71 2 (12.5) 100
 50 0.00 100.00 100.00
 100 0.00 100.00 100.00

[illegible]

73 Both α and β gene transfer experiments, especially in vascular
cells, have been difficult. A new method of gene transfer into this
tissue would be extremely useful. Previous methods have either
provided low levels of expression or require complicated manipulation
of viral vectors. The goal of this study was to develop an easy,
efficient method for transferring purified plasmid DNA into vascular
tissue. α and β gene transfer demonstrated that complexing unmodified
plasmid DNA with purified adenovirus (Ad5 dl312) via
electroporation *** lipid *** enhances gene transfer up to
100 fold in cultured bovine aortic endothelial cells (BAECs).
Further, in a large blood injured rabbit femoral artery model,
transfection of the gene in the neointimal smooth muscle cell
layer after balloon injury was demonstrated following transfection with a
plasmid encoding α and β gene and the SV40 ***nuclear***
localization signal *** control ***. Control arteries demonstrated
no detectable α or β . Our studies suggest that complexing plasmid

1. A third mechanism by which the virus might enhance gene transfer both in vivo and in vitro would have a wide range of applications for experimental vascular tissue.

= supply lysine and a few more

5 31 MAY 1968
1 12 50.4 LAST E
(CLUT) (CLUT) LAST E
2 9811 12 50.4 LAST E

IDENTIFICATION

$$= \frac{d}{dt} (\log b) + \frac{d}{dt} (\log a)$$

I : SWE 10 3 T E
 A : 146 39 EDITFe
 T : tel: 871 1 1 1 mediated gene delivery by
 I type 10
 A : C 1; Hial
 C : Department of Pharmacology, University of Pittsburgh School of
 Medicine 15261 USA.

N: 1 50 36 1 31
 (50 36 1 31
 1 50 36 1 31
 S: 1 50 36 1 31 1027-36.
 Juna 1962 7.00. 10. 00 360.

C: United States
 D: Journal; Author; JOURNAL ARTICLE
 L: English
 F: Biology; Life Sciences
 E:

A3: We observed that several high molecular weight cationic polymers, such as ***poly*** (***lysine***) and protamine, enhanced the transfection efficiency of several types of cationic liposomes 1-2 fold in a number of cell lines in culture. Small peptides such as spermine and a cationic decapeptide were also found to be either weakly or moderately active. The addition of ***poly*** (***lysine***) and protamine did not increase the particle size of the complex formed between DNA and cationic liposomes and rendered DNA resistant to the nuclease activity. The complexes composed of DNA, ***poly*** (***lysine***), and cationic lipids were found to be more effective in transfecting cells with sucrose gradient ultracentrifugation. The lipid complex formed at low cationic

liposome content and only had weak transfection activity. Addition of free liposome to the lipid-DNA complex significantly enhanced the transfection activity. Complexes formed at a higher initial ratio of liposome to DNA had a higher lipid content and were highly active in transfection. The complexes were about 3-9-fold more active than the complexes prepared after purification. Negative stain electron microscopy showed that the non-active complexes prepared from 40:1 lipid to DNA (0.5:1 ratio) were ***poly*** (***L*** - ***S***), and the complexes of DNA and purified by gradient ultracentrifugation were spherical, electron dense, small (< 100 nm diameter), and some of them were associated with lipid membrane. The highly active, stable, small-sized lipid/DNA complexes (***poly***)/DNA complexes represent a new class of delivery vehicles that might be useful in gene therapy.

LA ANSWER 2001 MEDICAL

AN 935976 POLINE

TI Large polypeptide carrier with a low molecular weight glycopeptide carrier.

AU Vithw M S, Knoll D J, Borge A P, Rice K G

CO College of Pharmacy, Ohio State University, Columbus 43210, USA..

NI D 141 (N 01)

G 18 1 1 1 1

SO J OCCUPATIONAL MEDICINE 99 (Mar 1998) 6 (3) 283-91.

Summary of the article is available in the following

CM United States

DI Chemical Abstracts (CODEN) 1998

LA English

FB Polypeptide carrier

EM 11

AB A large polypeptide carrier with a low molecular weight glycopeptide carrier was prepared by coupling a glycopeptide carrier with a large polypeptide carrier to dp19 ***poly*** - ***L***. The glycopeptide carrier was prepared by coupling in a 1:1 conjugate. The glycopeptide carrier complexed with plasmid DNA as evidenced by densitometry, ultracentrifugation, light scattering by condensed DNA, and mobility shift of condensed DNA on agarose gel electrophoresis. The glycopeptide carrier complex was taken into HepG2 cells via the endocytosis pathway. The transfection of recognition of terminal galactose residues on the glycopeptide carrier. The resulting luciferase reporter gene expression was dramatically influenced by the solubility of the glycopeptide carrier, the extent of conjugation, and the presence of the glycopeptide carrier. The results suggest that low molecular weight glycopeptide carrier might be suitable for further development as a delivery vehicle for receptor-mediated gene delivery.

CS Department of Pediatrics, University, Hamilton, Ontario,
Canada

SO H. A. ... 165-75.

... 1983

CY United States

DT ...

LA English

FS ...

EM ...

AB Human gene therapy protocols depend on genetically altered fibroblastic cells. We propose an alternate approach by implanting genetically altered fibroblast cell lines to deliver desired gene products in vivo. The recombinant allogeneic cells are implanted after implantation by enclosure within a microencapsulated ... ***poly*** - ***L*** - ... The clinical efficacy of this approach is being evaluated by implanting microencapsulated ... to secrete mouse growth hormone into the ... dwarf mice. The treated mutants ... body weights, peripheral organ ... significantly greater ... controls. Secondary response to the ... also resulted in increased ... first month post-implantation. The ... months of implantation ... fibroblasts retained a viability of ... growth hormone. Thus, ... recombinant cells corrected partially ... mutation in the ... cells remained functional for ... method of delivery recombinant gene products ... obviates the need for ... and is amenable to ... should have wide applications ... continuous supply of recombinant ...

L7 ...

AN ...

TI Gene ... of animals by targeting the ...

AU ... C S; Hanson R W; Davis P B

CS ... and Childrens Hospital,

NC ...

SO 1995 Feb) 95 (2) 493-502.
 CY 1995 Feb) 95 (2) 493-502.
 DT 1995 Feb) 95 (2) 493-502.
 LA 1995 Feb) 95 (2) 493-502.
 FS 1995 Feb) 95 (2) 493-502.
 EM 1995 Feb) 95 (2) 493-502.
 AB Gene therapy is a novel approach to the treatment of genetic diseases. In this study, we have developed a novel gene delivery system based on the use of a polymeric carrier, consisting of the Fab portion of a monoclonal antibody directed against rat secretory phospholipase A2 (sPLA2). This system was used to deliver a luciferase gene to the lungs of rats. The results show that the polymeric carrier system is highly efficient in delivering the luciferase gene to the lungs of rats, resulting in high levels of luciferase activity in the lungs. The results also show that the polymeric carrier system is highly specific for the lungs, with no significant activity in other tissues. These results suggest that the polymeric carrier system may be a useful tool for the delivery of genes to the lungs of rats, and may have potential applications in the treatment of lung diseases.

L7 1995 Feb) 95 (2) 493-502.
 AN 1995 Feb) 95 (2) 493-502.
 TI 1995 Feb) 95 (2) 493-502.
 AU 1995 Feb) 95 (2) 493-502.

CY GERMANY
DT
LA
FS
EM
AB

... leads to a
... and causes an increase in
... exchange and Na⁺, K⁺, 2Cl⁻
... transport systems are thought to be
... the aim of this study was to examine
... and cytoskeletal
... the oncogene. The experiments
... transfected with a
... and expressing the oncogene
... and 1 mumol/l dexamethasone
... expressing the oncogene (-ras
... but without the addition
... The growth characteristics
... architecture was visualized by
... using specific antibodies and
... was accompanied by
... increase in proliferative
... of the dishes with attachment
... **lysine** , collagen type
... slower on substrates
... **lysine** than on
... type I. Expression of the ras
... increase in cell volume
... protrusions and tended to
... Examination of the
... ras and -ras cells revealed marked
... of the stress fiber network
... as well as the absence of
... (focal contacts), a
... of cell surface fibronectin
... of microtubules and
... was also observed in
... fibroblasts which were not
... (ras cells) and which were also
... described above (low serum
... exhibit the cytoskeletal
... The results demonstrate that
... not only profound alterations
... volume and cell morphology, but
... skeletal architecture, which may

... of volume-regulatory ion
... ion

L7
AN
TI
... cells through use of
... to biotinylated transferrin

AU
CS
SO
...; Ariatti M; Hawtrey A
... of Medicine, University of
...
... (2) 165-74.

CY
DT
LA
FS
EM
AB
... developed for studying the transfer of
... receptor-mediated endocytosis.
... 460 was covalently
... of avidin via periodate
... avidin-pLys460. Following
..., the conjugate was reacted with
... avidin-pLys460-[bio-
... by Superose 12 gel
... experiments with DNA.
... avidin-pLys460-[bio-
... by nitro cellulose filter
... assays. The conjugate was shown
... complexes soluble in 0.15-0.2 M
... avidin-pLys460-[bio-transferrin]
... accomplished with Hela cells,
... melanoma cell line used in the
... was dependent on
... lysosomotropic agent
... with a receptor-mediated
... for Hela cells and a
... endocytosis for the alpha T3
...

L7
AN
TI
... of an adenovirus-based ligand
... transfer.
AU
CS
... Engineering, University of

SO EMBRYOLOGY JOURNAL, (1991 Apr 1) 49-58.
 Journal code: 9Y0. ISSN: 0264-6021.
 CY EMBRYOLOGY United Kingdom
 DT Journal Article; JOURNAL ARTICLE
 LA English
 FS Fertilization; Journal; Fertilization; Fertilization
 EM 910401
 AB Ligand-mediated approaches to gene transfer offer an alternative to viral vectors for both in vivo and in vitro applications. Although a significant proportion of the plasmid-based DNA complex is lost to lysosomal degradation following receptor-mediated endocytosis, simultaneous infection with a helper virus has been shown to increase the efficiency of gene transfer (Curriel, Agarwal, Wagner and Cullen (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 8850-8854; Wagner, Zoloth, Cotten, Hlappos, Mechtler, Curriel and Birnstiel (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 6099-6103]. In this study we describe an adenovirus-based ligand complex where the plasmid DNA, polycation-ligand complex and adenovirus are contained within a single particle. The ligand is a recombinant adenovirus encoding a DNA minigene for human placental alkaline phosphatase that was specifically modified with ***poly*** (***L*** - ***lysine***). Electron microscopy of an adenovirus-based ligand complex formed by associating plasmid DNA and an adenovirus with the ***poly*** (***L*** - ***lysine***) complex to give a single particle that appeared as intact viral particles coated with a dense biomolecular layer. Adenovirus-based ligand complexes containing either a luciferase or beta-galactosidase reporter plasmid were shown to efficiently deliver the transgene to cells that express the hepatic asialoglycoprotein receptor. Furthermore, the ***poly*** (***L*** - ***lysine***) complex greatly reduced the infectivity potential of the virus without causing a concomitant loss of augmented gene transfer. As an alternative to infectious virus, incomplete products of virus assembly were also considered as a means of gene transfer. However, these defective viruses were unable to significantly enhance plasmid transgene

L7 1991 OF 28 MEDICAL
 AN 1991 MEDICAL
 TI Clinical liposomes in vitro receptor-mediated
 *infectious***
 AU Nickerson, W. A. (1991)
 CS Department of Cell Biology, University of California-Davis..
 SO AMERICAN JOURNAL OF THE MEDICAL SCIENCES, (1994 Feb) 307 (2) 138-43.

cellular uptake of cells. We have previously shown that DNA molecules complexed with a viral envelope protein can be efficiently endocytosed by hepatocytes and the internalized DNA can be released and serve as a template for a replication-defective adenovirus. This adenovirus can then enter target cells if the infection agent by which the virus enters requires high concentrations of adenoviral particles. In this study, adenoviral particles were complexed with polyplexes to ***poly*** (***L*** - ***lysine***) and delivered to the liver. Quantitative delivery to primary hepatocytes was achieved with significantly reduced viral titers. This suggests that the ***poly*** (***L*** - ***lysine***) complex was included in the complex. The complexed virus was used to deliver a DNA vector containing coding sequence for the use hepatocytes, resulting in the expression of a functional factor IX in the culture medium. The results suggest that receptor-mediated endocytosis complexed with polyplexes as a delivery vector should permit the administration of genes for gene delivery into the liver for the treatment of genetic diseases.

L7 A.S.W. OF 3 LETTERS
AN 9 7 01
TI A. ... specific for G-protein coupled
... as ... type tagged BLR1-transfected
293 ... cell ELISA
AU P.O. Box 100, E. ... Voss, C., ...
CS In the ... Martinsried, FRG..
SO B. ... COMMUNICATIONS, (1993 Nov 15)
1 ...
CY ...
DT ...
LA ...
FS ...
EM ...
AB M. ... G-protein coupled receptors are
... ELISA-based screening system
for ... the G-protein coupled
receptor BLR1 ... (1992, 12:2795) using human embryonic
kidney ... modified human BLR1 cDNA
... tagged BLR1 protein. Lou/C
... tagged 293 cells and
... 8.653 myeloma cells
... specific antibodies by comparing
to ... cells and to untransfected
cells ... - ***L*** -
* ... Cells were fixed with 2%

... (15) ... of erythropoiesis. Thus, a ... receptor-mediated ... which should be widely applicable for ... receptor systems both in vitro and in vivo.

L7 ABSTRACT OF ...
 AN 9 ...
 TI ... a new delivery system for ...
 AU ...
 CS ... of Toronto, Ontario, Canada..
 SO ...

CY ...
 DT ...
 LA ...
 FS ...
 EM ...

AB The ... gene therapy has been examined ... yet its slow progression ... attributed to problems associated with ... efficient gene transfers, to obtain ... the transfected gene, and the ... transplantation. Here we report ... therapy that overcomes all ... genetically modified cells in a ... introducing a system that can produce ... desired gene product. As a model, ... the human growth hormone gene (... - ***poly*** - ***L*** ... long-term in vitro studies ... cells was physiologically comparable ... the cells. Furthermore, there was ... the human protein by the ... steady increase in the ... by each cell, followed by a ... the feasibility of ... two study: Balb-c mice ... had detectable serum ... for the duration of the study ... recovered from a recipient ... continued to secrete high levels of ...

L7 ABSTRACT OF ...
 AN 9 ...

TI H... cement of receptor-mediated
 AU C... y hepatocytes.
 CS D... College of Medicine, Houston, TX
 7
 NC H...
 D...
 SO P... SCIENCES OF THE UNITED STATES
 C... 6.
 CY U...
 DT J...
 LA F...
 FS P...
 EM 90)
 AB W... DNA delivery system with the
 en... and shown that DNA can be
 del... resulting in a high level of
 g... conjugated with ***poly***
 (... to deliver the Escherichia
 coli... hepatocytes through
 b... protein receptor, only a low
 l... with less than 0.1% of
 t... This level of activity can be
 g... of the DNA.protein complex
 w... resulting in 100% of the
 h... -4-chloro-3-indolyl
 b... of beta-galactosidase
 e... enhancement of activity. To test
 t... liver system for the correction of
 p... that causes severe mental
 r... the human phenylalanine
 h... derived from a PAH-deficient
 m... reconstitution of enzymatic
 a... for efficient gene
 d... of hepatic disorders.

L7 A...
 AN 9-
 TI S... tosylated ***poly*** -
 *... cells.
 AU M... J; Mayer R; Monsigny M; Roche
 A...
 CS D... ugues et Lectines Endog`enes,
 C...
 SO N... 21 (4) 871-8.
 C... 3-1

t... op... human hepatoma cells. 4 d
 a... it... or core gene expression
 c... were measured to be 16
 n... respectively. Antigen production
 wa... of an excess of
 asialoglycoprotein
 r... SK-Hep1, did not produce any
 v... after incubation with HBV
 D... of asialoorosomucoid and
 *... Using a complete HBV
 ge... reached 16 ng/ml and 16
 U... Southern blots revealed
 c... including 3.5-, 2.4-, and 2.1-kb
 f... HBV DNA sequences
 i... single stranded forms were
 d... Finally, 42-nm Dane
 p... medium were visualized by
 el... demonstrates that a targetable DNA
 c... in vitro resulting in the
 p...

L7 A...
 AN 9...
 TI C... delivery and expression of
 e... modified ***poly*** (
 *... conjugate in mouse lung
 e...
 AU T...; Huang L
 CS D... of Tennessee, Knoxville..
 NC A...
 SO E... (Jul 15) 1131 (3) 311-3.
 C...
 CY M...
 DT C...
 LA E...
 FS F...
 EM 9...
 AB A... gene delivery and expression
 i... efficiency of N-terminal
 n... (**lysine***) (NPLL)
 c... antibody 34A can be improved by
 a... cationic liposomes.
 D... liposomes form a ternary
 e... the ability to bind
 s... the same time the addition of
 l... transfection*** efficiency of
 a... by 10 to 20-fold in mouse

L7 A...
AN S...
TI T... be ... and sperm cells.
AU I... Spadafora C
CS D... University La Sapienza, Rome,
SO M... (1992 Mar) 31 (3) 161-9.
CY U...
DT C...
LA E...
FS F...
EM S...
AB Exogenous plasmid DNA, showed a specific interaction with the exogenous nucleic acid. We have studied the kinetics of such interaction. Exogenous DNA is taken up by sperm cells within 15- to 20-min period and is specifically localized in the head area of the sperm head. DNA uptake is blocked by other polyanions as heparin and chondroitin sulfate. ***L*** - the uptake. DNA molecules of large size are taken up as compared to small ones. 60-80 kD proteins were also taken up and concentrated for NA at the same level. These data strongly suggest a specific interaction between foreign molecules and sperm head. On the basis of SDS-PAGE analysis, a protein(s) of 30-35 KD is involved in the exogenous DNA binding. Moreover, we have demonstrated that plasma contains factor(s) which has a powerful inhibitor effect on DNA uptake. The binding protein for the DNA and the experimental support the existence of specific factors, the sperm-DNA

L7	Author	MEIN V V
AN	9 835	1979
TI	Effect of ... on the ... transport of a ...	
AU	F ... Serebryakova N V; Murav'ev V P	
CS	Department of ... Ministry of Health, Moscow..	
SO	... (1979) ... 199 (2) 323-9.	

CY United States
 DT Chemical and Biological Sciences
 LA Enzymes
 FS Polymers
 EM
 AB A plasmid carrying the gene of the
 S... ***poly*** - ***L*** -
 *... selectively transfect PLC/PRF/5
 human pancreatic carcinoma cells with insulin receptors.
 *... efficiently competed by excess free
 insulin. In order to improve transport of the construct, it was
 fused to a... on and in cells
 via... and quantitative confocal
 laser scanning microscopy. At 37 degrees C, the labeled
 construct... intracellular acidic
 compartments. The subcellular location of fluorescence localized
 to... binding, endocytosis, and nuclear
 localization. These processes could all be competed by
 excess free insulin. The entry of the conjugate
 into the cell... by the insulin receptor.

L7 A... 23...
 AN 9...
 TI D... functional gene-delivery
 a... or ethidium homodimer to
 the...
 AU W... H; Birnstiel M L
 CS F... Vienna, Austria..
 SO F... (4) 226-31.
 C... A...

CY United States
 DT Chemical and Biological Sciences
 LA Enzymes
 FS Polymers
 EM
 AB V... transferrin-polycation
 conjugates... for the introduction of
 genes... here a more specific method
 for... DNA-binding compounds involving
 a... moiety. We used the
 p... (**lysine**) or the DNA
 binding... DNA-binding domains. Successful
 transfection... and expression of the
 protein... cells has been shown with
 the... activity of the
 transfected... conjugates is low relative to
 the... because of incomplete

delivery with TfEtD is
 complexes of the DNA with TfEtD
 are prepared. The gene
 transferrin-polylysine
 conjugates containing
 described conjugates containing
 method facilitates the synthesis
 of conjugates.

L7
 AN
 TI
 AU
 CS
 SO
 CY
 DT
 LA
 FS
 EM
 AB
 transfection
 of Tennessee, Knoxville
 1065 (1) 8-14.
 poly
 conjugated to N-
 effective carrier to promote
 cultured mammalian cells. The
 contained an average of two
 L. Similar conjugates of the
 a similar
 that the degradation of
 activity. Unconjugated
 transfection activity
 with respect to the DNA
 and the presence
 binding of lipopolylysine
 retardation of DNA in agarose
 at the optimal
 transfection, all DNA were
 The
 lipopolylysine, under optimal
 higher than that of lipofectin,
 over, lipopolylysine mediated
 of 10% calf serum; whereas
 activity under the same
 transfection
 on scraping the treated
 y transfected attached

100

[illegible]

L7 F OF A

AN E O

TI F E E Expression in vivo.

SO E. coli (S. 88-2).
 CY U. 100
 DT C. 100
 LA E. 100
 FS E. 100
 EM E. 100
 AB V. 100
 ing foreign DNA to hepatocytes
 that takes advantage of
 internalization. The idea
 possess a unique receptor that
 (asialo)glycoproteins. To
 bind DNA in a
 L -
 but noncovalent interaction.
 (AsOR), was chemically
 lysine) to form an
 lysine)
 to DNA were tested to
 content in a soluble complex
 To test the targetable
 L - ***lysine***
 pSV2 CAT containing the
 (CAT) driven by an SV-40
 a model system consisting of
 glycoprotein receptor (+)],
 and uterine smooth muscle
 as incubated with 0.2 micron
 lysine)-DNA
 plus AsOR, DNA plus
), or DNA alone. Cells were
 as a measure of gene
 smooth muscle [receptor (-)]
 chloramphenicol derivatives
 TRUNCATED AT 250 WORDS)

L7 Z. 100
 AN Z. 100
 TI Z. 100
 side chain lengths and of
 of basic
 cells to
 AU Z. 100
 SO Z. 100
 CY Z. 100

DT
LA
FS
EM
AB

cell sheets to
was determined for 5 basic
arobromide at 100 microng/ml,
for poly-L-ornithine of
nan of AMW 105000, and much
lysine of AMW 1700
uses were observed with the 2
much faster and sensitivity
polyornithines than for the
0 sensitized cells slightly
- ***lysine*** of AMW
ivity maximum. Analysis of the
L - ***lysine***
step 1 (attachment) and a

L7
AN
TI
AU
SO

ured mammalian cells. Binding
No. 13) 454 (3) 397-409.

CY
DT
LA
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were incubated for a short
ial DNA and
n), ***poly*** - ***L***
agents previously
transfection
by 10(-6)-1.5 by 10(6) cells
r DNA representing 10-30% of
In contrast, when the cells
solution approx. 0.5-5% of the
ore than 95% of the
be bound to the surface of
also shown that in the absence
ll-associated donor DNA was

$\Rightarrow d k..$

APR 96)

24 APR 96

L1 2 TRANSFECTION

L2 TRANSFECTED CELLS AND TRANSFECTION

L3 NUCLEAR LOCALIZATION SIGNAL AN

L4 ? NUCLEAR LOCALIZATION SIGNAL A

L5 NUCLEIC ACID POLYMERASE AND LIPOSOME

L6 17 ANIONIC, NEUTRAL AND CATIONIC LIPID?

L7 CTION

\Rightarrow Sp. 1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100. 101. 102. 103. 104. 105. 106. 107. 108. 109. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120. 121. 122. 123. 124. 125. 126. 127. 128. 129. 130. 131. 132. 133. 134. 135. 136. 137. 138. 139. 140. 141. 142. 143. 144. 145. 146. 147. 148. 149. 150. 151. 152. 153. 154. 155. 156. 157. 158. 159. 160. 161. 162. 163. 164. 165. 166. 167. 168. 169. 170. 171. 172. 173. 174. 175. 176. 177. 178. 179. 180. 181. 182. 183. 184. 185. 186. 187. 188. 189. 190. 191. 192. 193. 194. 195. 196. 197. 198. 199. 200. 201. 202. 203. 204. 205. 206. 207. 208. 209. 210. 211. 212. 213. 214. 215. 216. 217. 218. 219. 220. 221. 222. 223. 224. 225. 226. 227. 228. 229. 230. 231. 232. 233. 234. 235. 236. 237. 238. 239. 240. 241. 242. 243. 244. 245. 246. 247. 248. 249. 250. 251. 252. 253. 254. 255. 256. 257. 258. 259. 260. 261. 262. 263. 264. 265. 266. 267. 268. 269. 270. 271. 272. 273. 274. 275. 276. 277. 278. 279. 280. 281. 282. 283. 284. 285. 286. 287. 288. 289. 290. 291. 292. 293. 294. 295. 296. 297. 298. 299. 300. 301. 302. 303. 304. 305. 306. 307. 308. 309. 310. 311. 312. 313. 314. 315. 316. 317. 318. 319. 320. 321. 322. 323. 324. 325. 326. 327. 328. 329. 330. 331. 332. 333. 334. 335. 336. 337. 338. 339. 340. 341. 342. 343. 344. 345. 346. 347. 348. 349. 350. 351. 352. 353. 354. 355. 356. 357. 358. 359. 360. 361. 362. 363. 364. 365. 366. 367. 368. 369. 370. 371. 372. 373. 374. 375. 376. 377. 378. 379. 380. 381. 382. 383. 384. 385. 386. 387. 388. 389. 390. 391. 392. 393. 394. 395. 396. 397. 398. 399. 400. 401. 402. 403. 404. 405. 406. 407. 408. 409. 410. 411. 412. 413. 414. 415. 416. 417. 418. 419. 420. 421. 422. 423. 424. 425. 426. 427. 428. 429. 430. 431. 432. 433. 434. 435. 436. 437. 438. 439. 440. 441. 442. 443. 444. 445. 446. 447. 448. 449. 450. 451. 452. 453. 454. 455. 456. 457. 458. 459. 460. 461. 462. 463. 464. 465. 466. 467. 468. 469. 470. 471. 472. 473. 474. 475. 476. 477. 478. 479. 480. 481. 482. 483. 484. 485. 486. 487. 488. 489. 490. 491. 492. 493. 494. 495. 496. 497. 498. 499. 500. 501. 502. 503. 504. 505. 506. 507. 508. 509. 510. 511. 512. 513. 514. 515. 516. 517. 518. 519. 520. 521. 522. 523. 524. 525. 526. 527. 528. 529. 530. 531. 532. 533. 534. 535. 536. 537. 538. 539. 540. 541. 542. 543. 544. 545. 546. 547. 548. 549. 550. 551. 552. 553. 554. 555. 556. 557. 558. 559. 560. 561. 562. 563. 564. 565. 566. 567. 568. 569. 570. 571. 572. 573. 574. 575. 576. 577. 578. 579. 580. 581. 582. 583. 584. 585. 586. 587. 588. 589. 590. 591. 592. 593. 594. 595. 596. 597. 598. 599. 600. 601. 602. 603. 604. 605. 606. 607. 608. 609. 610. 611. 612. 613. 614. 615. 616. 617. 618. 619. 620. 621. 622. 623. 624. 625. 626. 627. 628. 629. 630. 631. 632. 633. 634. 635. 636. 637. 638. 639. 640. 641. 642. 643. 644. 645. 646. 647. 648. 649. 650. 651. 652. 653. 654. 655. 656. 657. 658. 659. 660. 661. 662. 663. 664. 665. 666. 667. 668. 669. 670. 671. 672. 673. 674. 675. 676. 677. 678. 679. 680. 681. 682. 683. 684. 685. 686. 687. 688. 689. 690. 691. 692. 693. 694. 695. 696. 697. 698. 699. 700. 701. 702. 703. 704. 705. 706. 707. 708. 709. 710. 711. 712. 713. 714. 715. 716. 717. 718. 719. 720. 721. 722. 723. 724. 725. 726. 727. 728. 729. 730. 731. 732. 733. 734. 735. 736. 737. 738. 739. 740. 741. 742. 743. 744. 745. 746. 747. 748. 749. 750. 751. 752. 753. 754. 755. 756. 757. 758. 759. 760. 761. 762. 763. 764. 765. 766. 767. 768. 769. 770. 771. 772. 773. 774. 775. 776. 777. 778. 779. 780. 781. 782. 783. 784. 785. 786. 787. 788. 789. 790. 791. 792. 793. 794. 795. 796. 797. 798. 799. 800. 801. 802. 803. 804. 805. 806. 807. 808. 809. 810. 811. 812. 813. 814. 815. 816. 817. 818. 819. 820. 821. 822. 823. 824. 825. 826. 827. 828. 829. 830. 831. 832. 833. 834. 835. 836. 837. 838. 839

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L8

$$\Rightarrow d \mid b_1$$

L8 A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

[illegible]

TI Potentiation of α - β T cell *** -mediated gene delivery
by

AU C₁ X

CS Debra L. Davis, Director of Pittsburgh School of

M... .. USA

NC F 2 1

C: ...)

114

SO E HF 7-31

[illegible]

CY 12 11 11

DT C A S AP ICL

LA E. 3

FS P r e s e n t a t i o n s

EM C

AB. We have also prepared a series of low molecular weight cationic polymers,

lysine***) and protamine,

of several types of cationic

[illegible]

L8 Z. J. F.
AN C. J. F.
TI M. J. F. * ** *** **** ***** Aggregation by polycationic

AU E. M. R.
CS D. S. University Health Sciences

SO I 19 15 .51 60.

[illegible]

LA P. 11
FS I. 11
EM S. 11
AB I. 11
LA P. 11
FS I. 11
EM S. 11
AB I. 11

v... .. per to hexagonal phase
 t... .. phosphatidylethanolamine, either
 d... .. 1:2-
 c... .. led the gel to liquid
 c... .. of 1:1 mixtures of
 p... .. ethanolamine, both in dimyristoyl
 f... .. forms, as a function of pH
 a... .. polycationic amino acids. We
 c... .. miscible at all pH
 v... .. off the polypeptides. However,
 t... .. phase separation at higher pH
 a... .. peptide: as neither changes in curvature
 s... .. induced by the polycationic
 a... .. ability to induce leakage
 a... .. labelled with pyrene on one of
 t... .. emission from both monomer
 a... .. intensity from these two
 i... .. separation and the degree of
 i... .. molar mixtures of the
 c... .. and
 p... .. crystalline phase at 30
 c... .. the ratio of excimer to
 r... .. ***poly*** - ***L*** -
 e... .. of excimer emission from
 t... .. from 5 to 7. Poly-L-histidine
 i... .. ratio at pH 5 but not at pH
 r... .. expect for lateral phase
 s... .. the consequence of the
 p... .. diffusion of the lipids.
 e... .. over a range of
 t... .. both gel and liquid
 c... .. from the behaviour of the
 p... .. hard digitation. (ABSTRACT
 5 111

L8 7 4
 AN 9
 TI 5 use oxidase electrodes for the
 AU 5 son S P
 CS 9 City of Manchester, UK..
 SO 1 11 (3-4) 251-60.
 CY 1
 DT 1
 LA 1
 FS 1

EM 9
 AB 10 the measurement of glucose
 11 Glucose oxidase has been used
 12 sensors but the performance
 13 saturation kinetics, which
 14 relevant glucose
 15 limiting membranes have been
 16 of the enzyme to lower
 17 present in the bulk test
 18 electrode was reported whereby
 19 liposomes so that the lipid
 20 membrane. It was shown that the
 21 lipid constituents of the
 22 response to glucose could be
 23 fibres research undertaken to
 24 liposomal enzyme electrode.
 25 demonstrated with the use of
 26 solution. The variation in
 27 amount of glucose oxidase
 28 The new method allows a
 29 from a single batch of
 30 lipid
 31 the response of the electrode
 32

L8 33
 AN 34
 TI 35 by ***poly*** (***L***
 36 ***liposome*** encapsulation.
 AU 37 Alkand P; Lebleu B
 CS 38 Université Montpellier II
 39 St. Louis de France.
 SO 40 1; 31-94. Ref: 89
 41
 CY 42
 DT 43
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 LA 45
 FS 46
 EM 47

=> S 1

[illegible]

...ion. Negative stain EM studies
...prepared from 40 nmol of
L - ***lysine***
...gradient
...electron dense, small (< 100 nm
...were associated with lipid
...small-sized lipid/
.../DNA complexes represent a
...that might be useful in

L9
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...between ***cationic***
...DNA.
...J; Graham R W; Bally M B
...Columbia Cancer Agency,
...
...
...
...
...lipid-based carrier system for
...requires the characterization of
...and carrier formation. We
...can be formed following
...lipids*** to DNA in a Bligh
...chloroform/methanol/water
...the monophasic into a two-phase
...into the organic phase. When
...***lipids***, such as
...dioleoyldimethylammonium
...methanaminopropane chloride,
...can be recovered in the organic
...concentrations sufficient to
...the polyvalent
...dioleoyloxy-N-
...thyl - 1- propanaminium
...spermidine are used,
...the organic phase is also
...lipid and DNA is
...hydrophobic DNA complex can
...***lipids***. In the
...of excess Ca²⁺,

lysine), 100% of
The monovalent
can also be prepared
concentrations of NaCl (<
Importantly, these
lipid
The methods described,
characterized in the
ACT (UNDATED AT 250 WORDS)

[illegible]

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1. The beta gene, i.e. a PU box
 2. section*** of PU.1 in B
 3. at macrophages represses I-A
 4. of PU.1 was obtained
 5. ***domain*** of the
 6. with the interaction domain. Using the gel
 7. in vitro
 8. bind to the Y box of the I-A
 9. the assay, a supershifted DNA
 10. IFY proteins bind to the
 11. beta gene expression is
 12. of the domain.

L11 1. expression of GAL4 fusion
 AN 2. tail.

AU 1. E; en
 CS 1. Charlestown Hospital, Charlestown

SO 1. 291-8.

CY 1.
 DT 1.

LA 1.

FS 1.

EM 1.

AB 1. human cell lines is an
 2. of the proteins encoded
 3. however, evaluation of the
 4. difficulty in purification of
 5. of specific antibodies which
 6. for immunocytochemistry
 7. fusion systems have been
 8. to the expression product
 9. purified using affinity
 10. by immunocytochemistry
 11. affinity handle
 12. ***binding*** ***domain***
 13. is widely used to construct
 14. factors to evaluate
 15. the lack of commercially
 16. other biochemical
 17. has remained difficult. We
 18. human expression vectors,
 19. for pm2, Flag, Histidine
 20. of the yeast

the cr... peptide (consisting of the
the 10 product from
a tail of six histidines
lines allow both the
the DNA-binding domain and
the... with another insert. (ABSTRACT
NAT...)

L11
AN
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AB
... gene, which plays an
... characterized the
... (QNR) gene. In addition to
... revealed several putative
... three myb-responsive elements
... binding phosphoprotein that
... ***transfection*** in
... QNR... promoter with a vector
... resulted in an increase in
... experiments we identified
... within the promoter
... binding domain fused to the
... efficient in Pax-QNR promoter
... can transactivate through a
... truncated ***protein***
... ***domain*** was also
... These results show that
... the myb protein directly as
... situ hybridization that
... neuroretina,
... these observations suggest that the
... Pax-QNR/pax-6.

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